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THE INFLUENCE OF SIZE ON THE RADULA OF *LITTORARIA ANGULIFERA* (GASTROPODA: LITTORINIDAE)

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ABSTRACT

We evaluated the radula shape of *Littoraria angulifera* from mangroves and rocky shores on the Brazilian coast. We compared snails sampled in a mangrove and on a rocky shore located about 100 m apart, and also from locations hundreds of km apart. Smaller individuals sampled on rocky shores have elongated cusps, whereas larger individuals have subequal cusps, similar to those observed in mangrove specimens. Distinct factors in each environment might exert different effects on the radula shape of *L. angulifera*. The greater morphological diversity observed on the rocky shores might be due to the heterogeneity of algae composition and partitioning of resources, a hypothesis that can be tested in further studies.

Key words: *Littoraria angulifera*, mangroves, phenotypic variation, radula, rock substratum.

INTRODUCTION

Phenotypic variation in radulae has been studied in several littorinid species because of its intrinsic relationship with diet (Padilla, 1985, 1989, 1998; Padilla et al., 1996; Trussell, 2000; Ito et al., 2002). Differences in the morphology of this structure have been related to sexual dimorphism (Fujioka, 1985; Mutlu, 2004), diet (Padilla, 1998; Padilla et al., 1996), temporal and spatial variations in substrata (Reid & Mak, 1999; Ito et al., 2002), size (Isarankura & Runham, 1968), and age (Nybakken, 1990; Kawamura et al., 2001). The radula of littorinids is of the taenioglossate type, characterized by a central rachidian, surrounded by a pair of lateral teeth, a pair of inner marginal teeth, and a pair of outer marginal teeth. The functional significance of the form of radular teeth is still unclear, but it is probably closely associated with feeding habits and, consequently, with the environment (Padilla, 1985, 2004; Padilla et al., 1996; Reid, 1996). The radula has been used as an important source of characters in gastropod systematics, both for taxonomy and for phylogenetic reconstruction. Evaluation of intraspecific variation in radular form can, in some cases, bring about an understanding of its cause.

Littoraria Griffith & Pidgeon, 1834, is a genus with 39 known species that occur in mangroves, salt marshes, and rocky shores (Reid 1986, 1999, 2001). Two species are found along the Brazilian coast: *L. flava* (King & Broderip, 1832), usually restricted to rocky shores near freshwater, but also found in mangroves (Reid & Mak, 1999), and *L. angulifera* (Lamarck, 1822), which occurs in mangroves and, occasionally, on nearby rocky shores (Gallagher & Reid, 1974). Kohlmeyer & Bebout (1986) suggested that the diet of *L. angulifera* was based on fungus-infested plants and fungi. Reid & Mak (1999) reported differences in the radular form of this species collected on rocky shores and on mangroves that are probably related to environmental conditions. In this study, we compared radula from *L. angulifera* individuals collected on mangroves and rocky shores in different locations, trying to assess if such variation could be observed in Brazilian samples.

MATERIALS AND METHODS

Part of the samples was conducted on Dura beach (23°29'S, 45°10'E), a sheltered location in the Escuro River estuary at Ubatuba. At this location, both environments are present, separated by about 100 m – a mangrove along the estuary and a salt-water rocky shore. Snails were collected from both environments. The mangrove vegetation at the Escuro River consisted mainly of three species, the marsh grass *Spartina* sp., and *Avicennia schaueriana* and *Laguncularia racemosa*, very common shrubs in the mangroves of southeastern Brazil. The sampling was done on February 20, 2003. Three samples of ten individuals were collected: on the rocky shore, on *Spartina* blades, and on the stems and aerial roots of *L. racemosa* and *A. schaueriana*.

Radulae from all 30 individuals were studied, but only 21 resulted in good images for electronic microscopy and were actually photographed. In the laboratory, the snails were immersed in 7.5% MgCl solution and then fixed in 70% ethanol. The shell heights (maximum dimension parallel to the axis of coiling) were measured to the nearest 0.01 mm with a digital caliper. To isolate the radula, the snails were kept in 7.5% KOH at 50°C until the soft body was completely dissolved (10–20 min), followed by gentle cleaning with a fine paintbrush and rinsing with distilled water. (In a previous study, heating did not cause any change to the radulae, compared with non-heated samples.) The radulae were mounted flat on aluminum stubs and held in place with dual adhesive tape. The structures were coated with gold and examined by scanning electron microscopy (SEM). Only fully formed and unworn teeth from the central third of the radula ribbon were examined. The sample size used for SEM analysis and the range of shell height are shown in Table 1. Photographs were taken in two standard orientations: at 45° from the front end of the radula and at 45° from the side of the radula, to show shape of tooth cusps and relief.

Radulae from periwinkles collected on rock and mangrove substrates from distant locations were also compared. Eighteen individuals collected on Mangaratiba mangrove shrubs (southeastern coast; 22°56'S, 44°4'E; September 1998) and 13 specimens from the rocky shore in Santa Barbara Island, Abrolhos Archipelago, (northeastern coast; 18°21'S, 38°36'E; October 1998) were also examined using a light microscope.

The description of the radula characters was based on Reid & Mak (1999); in our study, the major cusps are referring only to the lateral and inner marginal teeth. The counting of cusps was restricted to 4–20 rows for each individual based on photos of SEM or as seen in a light microscope. The number of cusps was counted on all visible teeth on both left and right sides of each radula. The characterization of the form of the major cusp (relative to the smallest cusps) on lateral and inner

TABLE 1. Specimens of *Littoraria angulifera* examined with SEM and light microscopy. The form of the major cusps of the lateral and inner marginal teeth and the number of cusps are shown for each sampling site (see text, Fig. 1 for explanations). The number of cusps considered was the observed on the 4–20 rows analyzed in the third central part of the radulae. The sample size is shown in parentheses. SH = range of shell height (mm). As "shrubs" we indicated the samples collected on *L. racemosa* and/or *A. schaueriana*.

Samples	SH	Form of major cusps	Lateral teeth	Inner marginal teeth	Outer marginal teeth
Dura Beach					
Rocky shore, stone (5)	6.7–9.4	elongate	5	4	4, 5
Rocky shore, stone (3)	14.6–18.0	subequal	5	4	5, 6
Mangrove, *Spartina* (4)	6.7–10.1	subequal	5, 6	4	6
Mangrove, *Spartina* (3)	10.9–12.6	subequal	5, 6	4	6
Mangrove, shrubs (6)	11.1–17.1	subequal	5, 6	4	6
Mangaratiba					
Mangrove, shrubs (11)	5.9–9.7	subequal	5, 6	4	5, 6
Mangrove, shrubs (1)	6.4	elongate	5	4	5
Mangrove, shrubs (6)	10.3–22.0	subequal	5, 6	4	6
Santa Barbara Island					
Rocky shore, stone (9)	5.2–10.7	elongate	5	4	4
Rocky shore, stone (4)	13.0–22.8	subequal	5, 6	4	4, 5

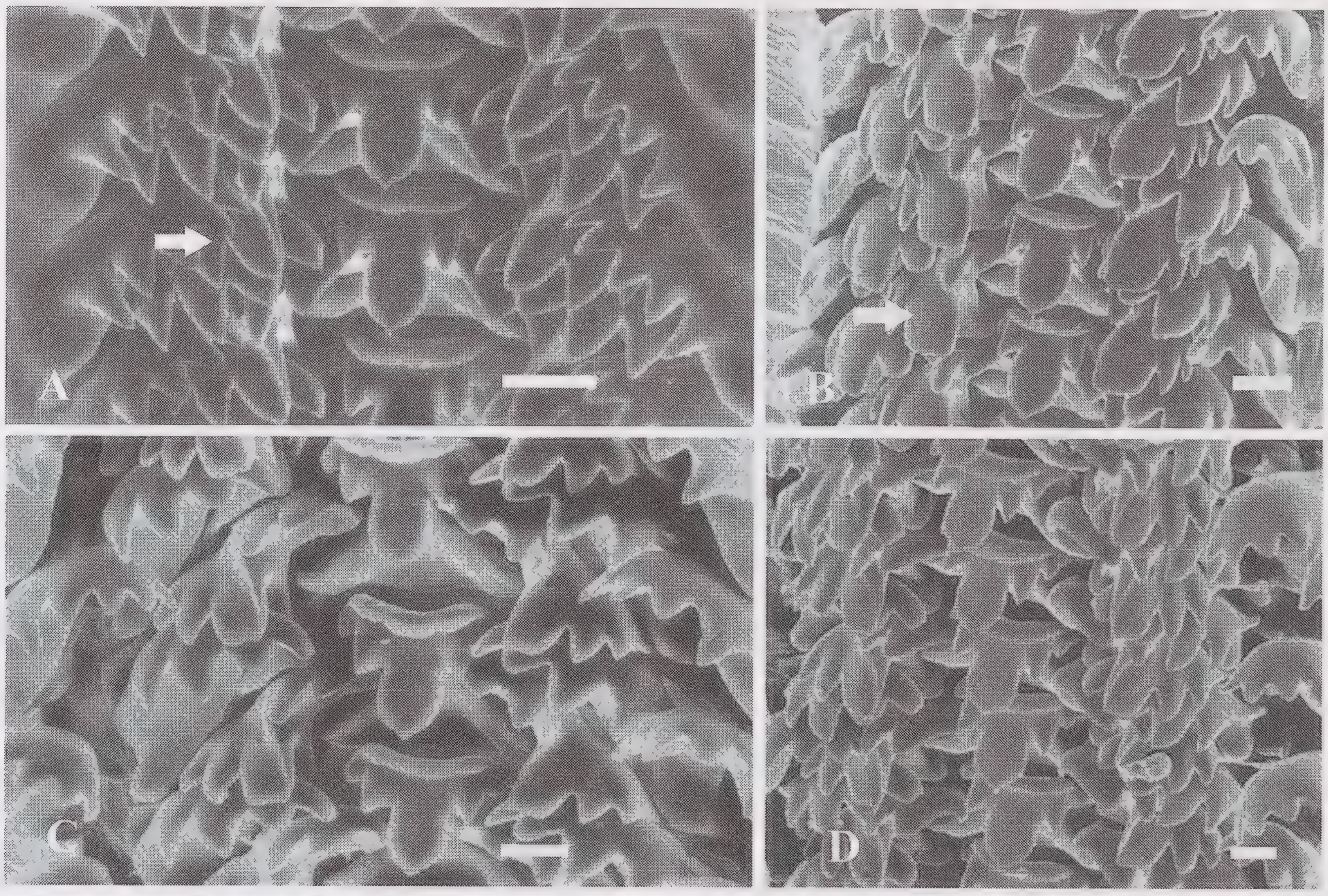

FIG. 1. Radulae of *Littoraria angulifera* samples. A: Rocky shore (shell height = 14.6 mm), lateral teeth subequal (arrow), at 45° from front; B: Rocky shore (shell height = 6.7 mm), lateral tooth elongate (arrow), at 45° from side; C: Mangrove, on *Spartina* (shell height = 10.9 mm), at 45° from front; D: Mangrove, on shrubs (shell height = 17.0 mm), at 45° from side. Scale bars = 10 µm.

marginal teeth was assigned in the following categories: subequal, characterized by sharper, more uniform cusps (e.g., Fig. 1A), and elongate, characterized by larger, blunt cusps (e.g., Fig. 1B).

To evaluate the influence of size on the shape of cusps, we performed a logistic regression. The dependent variable was the shape, codified as subequal (0) or elongate (1). Here, the maximum-likelihood method was used to fit a regression line to a logit-transformed data, since the dependent variable was binomially distributed (Sokal & Rohlf, 1995: 767–778).

RESULTS

The radulae in all analyzed individuals had a large hood in the all environments with similar number of cusps in the outer marginal teeth in both mangroves and rocky shores (Table 1, Fig. 1). Among individuals, there was variation in the number of cusps in the lateral and outer marginal teeth, but with no evident pattern (Table 1). On the other hand, there was no intra-individual variation in the number of cusps in the analyzed rows.

Littoraria angulifera showed little differentiation in the shape of the major cusps between environments (Figs. 1A, C), even among the different locations. Among specimens from the rocky shore in Dura and Santa Barbara, individuals with different shell heights showed variation in the cusp shape on the lateral teeth (Figs. 1A, B). All individuals with a shell height equal to or less than 10 mm had blunter, larger cusps in lateral and inner marginal teeth than found on specimens with greater shell height (Table 1, Fig. 1B). In mangroves, this variation between small and large periwinkles was not observed (Figs. 1C, D), except for one specimen at Mangaratiba (Table 1).

Shell length had a significant association with cusp shape for snails from Dura and Santa Barbara rocky shores (logistic regression analysis: χ^2 = 10.58 and 15.9, d.f. = 1, p = 0.0011 and 0.00007, respectively). There was no similar relationship for individuals from Mangaratiba (χ^2 = 1.99, d.f. = 1, p = 0.15). There were no differences in the shape of the radulae among snails collected on shrubs and *Spartina* (Figs. 1C, D). Thus, the regression analysis was not performed for these individuals.

DISCUSSION

Reid & Mak (1999) reported differences in the radular form in *Littoraria* species collected on rock and plant substrata: individuals from the rocky shore had a markedly enlarged major cusp on each of the five central teeth and fewer cusps on the outer marginal teeth, compared with radulae of conspecifics from mangroves. We observed no difference in the shape of the radula between large snails from these two environments among all locations, in contrast to the observations of Reid & Mak (1999). The samples used in their study were collected in different places (Belize and Florida; D. G. Reid, personal communication), which could explain the variation observed. Our samples were collected at geographical distances ranging from 240 km (between Dura and Mangaratiba) to 1,160 km (between Dura and Santa Barbara). There was also temporal variation among samples. Even though our samples were collected from very distant locations, cusp shape varied less than those analyzed by these authors. There was only a slight difference between the mangrove and rocky shore samples in the number of lateral and outer marginal cusps (Table 1). Thus, it is possible that these differing findings might reflect interpopulational genetic variation, assuming that radular variation is a heritable character.

On the rocky shore, the cusps of the lateral teeth varied in shape depending on the size of the snails; the smallest individuals had elongated cusps. This could represent a normal ontogenetic process, as observed in *Conus* (Nybakken, 1990), but we do not know if the small periwinkles sampled were young adults or juveniles. Only one small individual in the Mangaratiba mangrove had elongated cusps, which could be explained by a low frequency of individuals bearing this cusp shape. In Mangaratiba, mangrove and rock substrata are very close to each other (50–80 m), and this individual could have moved to the mangroves immediately before sampling. Possible explanations of the variation observed in the rocky shore are: (i) individuals of different sizes from rock substrata may have distinct diets and may partition resources; (ii) this pattern results from an ontogenetic effect, which is dependent on environmental characteristics; (iii) distinct factors in each environment may have exerted different selective effects on radula shape in *L. angulifera*; and (iv) an interaction among these factors could have contributed to the divergent effect seen in these two environments.

Merkt & Ellison (1998) noticed that several morphological traits of *L. angulifera* also vary in response to fine-scale environmental conditions. These authors observed that morphological variation in some traits, such as shell shape, sculptural, and genital morphology, could be ascribed to habitat characteristics and environmental heterogeneity.

In a previous study at Dura Beach, we observed that *L. flava*, which inhabits the same environments as *L. angulifera*, showed variation in radular tooth shape and length (Andrade & Solferini, 2006). Snails collected on mangroves had elongate teeth, whereas specimens from mangroves had sharper, subequal cusps. This variation is expected in snails in general, because sharper and pointed teeth as observed in mangroves and rocky shore large individuals have less contact with the surface and are more effective at piercing and tearing fleshy algae, whereas elongated teeth seem to be more effective for rasping and removing loose material from surfaces or broad excavations of brittle materials, such as calcified algae (Padilla, 2004). However, no influence of size was observed in individuals of *L. flava* sampled in rocky shore and mangroves (Andrade & Solferini, 2006). This finding indicated that the environments might exert different effects on the radulae of this species. The diet of *L. flava* has not been investigated, whereas *Littoraria angulifera* has been described as a generalist, because its diet seems to be very diverse (Kohlmeyer & Bebout, 1986). Feeding choice in gastropods is not a simple issue and depends of several factors, such as local abundance, chemical defenses, nutritional income, among others (Padilla, 1985, 1989). Thus, it is likely that the diverse flora found on the rock substrata (and maybe also the mangroves) allow *L. angulifera* to partition resources. In this species, the greater heterogeneity of the rocky shore, with the availability of different substrata, compared with mangroves, could have resulted in a greater morphological diversity. More comprehensive experiments isolating ontogeny and environmental effects on littorinids are necessary to help solve this question. Additional experimental work will be important for understanding the processes leading to the definition of the morphological traits in littorinids.

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THE GASTROPOD COMMUNITIES IN THE LOWLAND RIVERS OF AGRICULTURAL AREAS - THEIR BIODIVERSITY AND BIOINDICATIVE VALUE IN THE CIECHANOWSKA UPLAND, CENTRAL POLAND

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ABSTRACT

Lowland river degradation of agricultural areas is a problem not only in Poland but also in Europe and worldwide. The objectives of the present study were to determine the biodiversity of the gastropod communities in lowland rivers under the impact of agriculture, the relationship between gastropod communities and environmental factors, and to estimate the usefulness of the gastropod communities as an indicator of water quality. Four rivers that flow through an agricultural area were investigated. A high value of chlorophyll *a*, a low value of total dissolved oxygen and a high concentration of phosphates or total phosphorus and organic matters are present in the rivers. Gastropod communities in the rivers of the Ciechanowska Upland are influenced by bottom sediments, the physical and chemical parameters of water, and the abundance of macrophytes, which are typical of eutrophic water. In the rivers of the Ciechanowska Upland, gastropods can be biological indicators of dissolved oxygen in water – *Viviparus viviparus* (Linnaeus, 1758) and *Lymnaea peregra* (O. F. Müller, 1774) can be indicators of anthropopressure. The values of diversity indices varied from site to site, but did not decrease along the river.

Key words: gastropod communities, lowland rivers, biodiversity indices, agricultural areas, biological indicators, bottom sediments, *Viviparus viviparus* (Linnaeus, 1758), *Lymnaea peregra* (O. F. Müller, 1774).

INTRODUCTION

The cleanness of rivers reflects all human activities within a catchment (Hildrew, 1996). In the rivers of agricultural areas, high concentrations of phosphates or nitrates accelerate the development of phytoplankton, diminishing macrophytes and increasing deficits in dissolved oxygen (Thiébaut & Muller, 1999). It has been estimated that in inland waters, 70% of the total phosphorus comes from surface flow from agricultural areas, 24% comes from municipal sewage, and 19% from detergents (Parr & Mason, 2003). Most rivers in agricultural areas have high concentration of total phosphorus, phosphates, nitrates, suspended solids, and pesticides. This includes such rivers as the Łydynia River, the Pełta River, the Sona River, and the Wkra River, which flow throughout the Ciechanowska Upland in central Poland. Lowland river degradation of agricultural areas is a problem not only in Poland but also in Europe (Solimini et al., 2000; Parr & Masson, 2003) and worldwide (Collier et al., 1998; Harding et al., 1999; Shieh et al., 1999).

Biodiversity assessments are frequently used to monitor the level of pollution (Sandin & Jochnson, 2000; Beaven et al., 2001). Diversity indices such as the Shannon-Wiener index and the Evenness index are based on the following assumptions: (1) there are fewer species in aquatic ecosystems under human impact compared to unpolluted water; (2) the diversity decreases with environmental degradation; and (3) values of the Shannon-Wiener index decrease with increasing pollution (Barton & Metcalfe-Smith, 1992). The objective of the present study were to determine the biodiversity of the gastropod communities in lowland rivers under the impact of agriculture, the relationship between gastropod communities and environmental factors, and to estimate the usefulness of gastropod communities as indicators of water quality.

MATERIALS AND METHODS

Study Area

The study area comprised 2,570 km^2 (the Ciechanowska Upland). The study was carried out in the years 1997–2000. Four rivers of the Ciechanowska Upland: the Łydynia River, the Pełta River, the Sona River and the Wkra River, which flow through this agricultural area, were investgated. Samples were gathered from the overall length of the rivers, but in the case of the Wkra River, only the middle and lower course were sampled (103 km) (Fig. 1). Ten sampling sites were chosen for the Łydynia, Sona, Wkra, and eight for the Pełta River.

The characteristic morphometric features of the rivers are shown in Table 1.

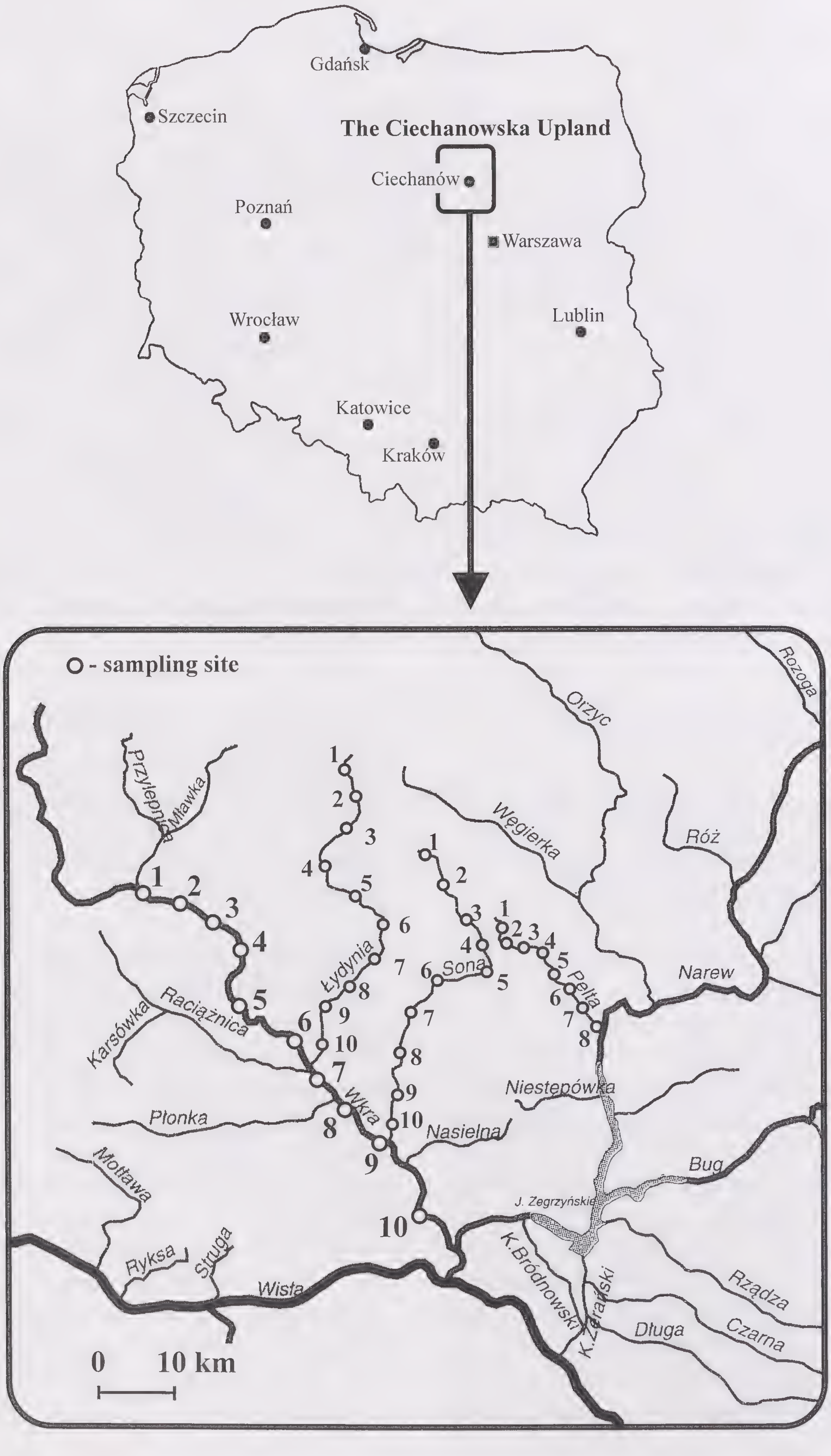

FIG. 1. Location of the study area (the Ciechanowska Upland).

TABLE 1. The morphometry of the rivers and the substratum.

River	Catchment area (in km^2)	River length (in km)	River width (in m)	Surface velocity (ranges) (m/s)	Substratum
Łydynia River	697.9	71.3	1.5–12.0	0.17–0.91	sandy-stony, sandy, stony, stony-sandy
Pełta River	368.7	50.7	0.7–4.0	0.03–0.3	sandy-muddy, sandy-stony, muddy-clay
Sona River	536.5	67.7	0.5–15.0	0.10–0.98	stony, stony-sandy
Wkra River	5,322.1	255.5	10.6–30.5	0.18–0.91	muddy-stony, sandy-muddy, sandy-stony

Methods

Gastropods were sampled by placing a quadrat frame (25 x 25 cm) on the substratum of the river. The frame was placed 16 times at each of the sample stations, which constituted one sample. At each sample station, samples were also taken of bottom sediments up to 5 cm, macrophytes and water surfaces. Only living specimens of gastropds were collected. Bottom sediments were taken by a core-type sampler. The collected material was brought back to the laboratory in plastic bags. The samples were than filtered using a 0.5 mm mesh sieve. The samples of gastropods were preserved in 75% ethanol. The species of most gastropods were identified according to Glöer & Meier-Brook (1998), whereas the Lymnaeidae were identified according to Jackiewicz (1998). The density of gastropods was estimated as the number of individuals per square metre. Immediately prior to gastropod sampling, water samples were collected from each sampling site. The analyses of the physical and chemical parameters of water, for example, temperature, pH, conductivity, dissolved oxygen, BOD$_5$, sulphates, chlorides, total dissolved solids, alkalinity, calcium, magnesium, ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, phosphates, total phosphorus, and chlorphyll *a*, were carried out according to Hermanowicz et al. (1976). Macrophyte species were recorded on the same visit as gastropod sampling. If macrophyte species could not be identified to species in the field, they were taken to the laboratory, dried between sheets of filter paper and after drying, mounted as ordinary herbarium species. Macrophytes were identified to species according to Szafer et al. (1986). The mineralogical analyses of the bottom sediments were carried out by means of a Siemens D 5,000 powder diffractometer. The organic matter content in the bottom sediments was determined according to Tiurin methods (Lityński et al., 1976). All samples were collected from the head to the mouth of rivers three times at each sample site. In total, 114 sites were sampled within the survey area. The zoocenological study of the gastropod communities was carried out using the following indices:

(1) Dominance (D%)

$D = n_a / n \times 100$

where n_a = the number of individuals of species a,

n = the total number of individuals in a sample.

The value of the dominance index D was divided into five classes according to Górny & Grüm (1981): eudominants > 10.0% of the sample, dominants 5.1–10.0% of the sample, subdominants 2.1–5.05 of the sample, recedents 1.0–2.1% of the sample, subrecedents < 1.0% of the sample.

(2) Constancy (C %)

$C = N_a / N \times 100$

where N_a = number of samples that contain species a,

N = total number of samples.

The value of the constancy index C was divided into four classes: euconstants 75.1–100.0% of the sample, constants 50.1–75.0% of the sample, accessory species 25.1–50.0% of the sample, and accedents ≤ 25.0% of the sample.

Biodiversity indices (Cao et al., 1996; Mouillot & Lepretre, 1999):

(1) The Shannon-Wiener index:

$H' = - \sum (P_i) (\log_2 P_i)$

where $P_i = N_i / N$ – the proportion of individuals belonging to species i.

(2) The Evenness index:

$J' = H' / \log_2 S$

where H' = the value of the Shannon-Wiener index,

S = the total number of species.

Correlation between gastropod density, number of species, organic matter content in the substratum, and the physical and chemical parameters of water were calculated by means of the non-parametric Spearman's Rank Correlation Coefficient r_S. Gastropod frequency in particular rivers in relation to the substratum was calculated by a chi-squared association test (χ^2) (Fovler et al., 1998).

Principal Component Analysis

The studied data sets contain measurement values of 18 various biological, physical and chemical parameters of water, collected at the sampling sites along the Pełta, Łydynia, Sona and Wkra rivers. The water parameters studied are listed in Table 2. The data are organized in matrices X (35 x 18). Each row of matrix X represents one sampling site, described by 18 parameters. Because the measured parameters significantly differ in their ranges, the data set was standardized according to the formula:

$$x_{ij} = \frac{(x_{ij} - \bar{x}_j)}{s_j}$$

where $\bar{x}_j, s_j$ denote the mean of the j-th column and its standard deviation, respectively. Depending on the organization of the data sets, different methods of exploratory analysis can be used. One of the most popular techniques of exploratory analysis of multivariate data sets is principal component analysis (PCA) (Wold, 1987; Massart et al., 1997). This technique allows reduction of data dimensionality, visualization and interpretation of the objects and variables relationships.

RESULTS

Physical and Chemical Parameters of Water and Macrophytes

The physical and chemical parameters of water and macrophyte species are shown in Tables 2 and 3.

A high value of chlorophyll *a*, a low value of total dissolved oxygen, and a high concentration of phosphates or total phosphorus and

TABLE 2. The physical and chemical parameters of water, organic matter content in the substratum, and their values.

Parameters	Łydynia River	Pełta River	Sona River	Wkra River
Temperature (°C)	18.2–20.0	14.8–20.0	15.1–19.5	17.5–19.1
pH	7.7–8.2	7.6–7.9	7.6–8.4	8.0–8.5
Conductivity (µS/cm)	490.0–610.0	691.0–771.0	390.0–681.0	450.0–620.0
Dissolved oxygen (mg O_2/dm^3)	1.60–8.80	6.30–14.10	4.6–10.4	4.50–8.60
BOD_5 (mg O_2/dm^3)	1.80–9.20	1.30–7.80	1.20–5.60	1.20–8.0
Sulphates (mg SO_4/dm^3)	60.0–71.0	42.0–80.0	34.0–79.0	27.0–65.5
Chlorides (mg Cl/dm^3)	23.0–29.0	20.0–51.0	15.0–23.0	19.0–22.0
Total dissolved solids (mg/dm^3)	320.0–372.0	438.0–613.0	280.0–488.0	295.0–369.0
Alkalinity (mg $CaCO_3/dm^3$)	200.0–245.0	290.0–370.0	165.0–210.0	195.0–285.0
Calcium (mg Ca/dm^3)	91.80–100.20	90.70–104.70	64.90–104.0	77.0–101.1
Magnesium (mg Mg/dm^3)	9.70–12.30	16.0–29.50	11.0–18.80	8.30–28.1
Ammonia nitrogen (mg N/dm^3)	0.30–3.74	0.86–1.98	0.06–1.16	0.27–0.92
Nitrite nitrogen (mg N/dm^3)	0.003–0.024	0.008–0.023	0.001–0.060	0.008–0.095
Nitrate nitrogen (mg N/dm^3)	0.07–2.11	0.25–0.60	0.05–0.99	0.06–1.40
Phosphates (mg PO_4/dm^3)	0.40–2.10	0.34–3.71	0.30–0.60	0.68–1.31
Total phosphorus (mg P/dm^3)	0.43–1.01	0.25–1.28	0.18–0.34	0.46–0.60
Chlorophyll *a*(µg/dm^3)	1.80–20.10	3.90–55.1	7.40–81.50	3.8–75.6
Organic matter (%)	1.17–11.86	1.72–28.44	0.43–16.43	0.86–11.10

TABLE 3. Macrophyte occurrence in the rivers of the Ciechanowska Upland.

Species	Łydynia River	Pełta River	Sona River	Wkra River
Acorus calamus L.			+	+
Alisma plantago-aquatica L.			+	+
Berula erecta (Huds.) Coville		+		
Butomus umbellatus L.	+	+		+
Ceratophyllum demersum L. s.s.				+
Elodea canadensis Michx.	+	+	+	+
Equisetum palustre L.				+
Glyceria maxima (Hartm.) Holmb.		+	+	+
Lemna minor L.	+	+	+	+
Lemna trisulca L.	+	+	+	+
Mentha aqatica L.			+	
Myosotis palustris (L.) L. em. Rchb.		+	+	+
Phragmites australis (Cav.) Trin. ex Steud.				+
Nuphar lutea (L.) Sibth. & Sm.	+	+	+	+
Potamogeton crispus L.	+			
Potamogeton natans L.		+		+
Potamogeton pectinatus L.				+
Potamogeton perfoliatus L.	+			
Rumex hydrolapathum Huds.				+
Sagittaria sagittifolia L.		+	+	+
Schoenoplectus lacustris (L.) Palla				+
Sparganium emersum Rehmann				+
Sparganium erectum L. em. Rchb. s.s.	+	+	+	+
Spirodela polyrhiza (L.) Schleid.			+	
Typha latifolia L.				+
Σ of species	8	11	12	20

organic matters were present in the rivers. The main sources of pollution were public utilities, agriculture and the food industry.

The organic matter in the bottom sediments ranged from 0.43% in the Sona River to 28.44% in the Pełta River (Table 2).

Zoocenological Study of the Gastropod Communities

In the rivers of the Ciechanowska Upland, 27 gastropod species were found (Table 4). In the Wkra River, the number of gastropod species was lower than in its tributaries, for example, the Łydynia River and the Sona River. The highest number of gastropod species were found in the Łydynia River. *Valvata piscinalis* (O. F. Müller, 1774) was eudominant in the communities in the Łydynia River and in the Pełta River; *Bithynia tentaculata* (Linnaeus, 1758) was eudominant in the Sona River, and *Viviparus viviparus* (Linnaeus, 1758) in the Wkra River. Both *Bithynia tentaculata* and *Lymnaea peregra* (O. F. Müller, 1774) were eudominants and euconstants in communities in the Łydynia River, whereas *Lymnaea peregra* in the Wkra River. Seven gastropod species were subrecedents as well as accedents of the gastropod communities (Table 4). In the Łydynia River, the values of the Shannon-Wiener index and the Evenness index ranged from 0 to 3.28 and from 0 to 0.98, respectively. At sites 9 and 10, the values of H' and J indices reached 0, because a drastic decrease in the number of gastropods species was observed there. The quantity ratio (the number of specimens of each species) was poorly balanced in the gastropod community at site 5 (dominance was focused on the one species mainly, for example, *Ancylus fluviatilis* O. F. Müller, 1774), thus the value of the Evenness index reached 0.6 (Fig. 2a). In the Pełta River the values of the Shannon-Wiener and the Evenness indices ranged from

TABLE 4. The values of the dominance (D) and constancy (C) indices (%) of the gastropod communities in the rivers.

Species	Łydynia River (n = 30)		Pełta River (n = 24)		Sona River (n = 30)		Wkra River (n = 30)	
	D	C	D	C	D	C	D	C
Viviparus contectus (Millet, 1813)	0.4	16.7	-	-	3.4	20.0	-	-
Viparus viviparus (Linnaeus, 1758)	-	-	6.7	4.2	1.2	6.7	25.6	67.9
Bithynia tentaculata (Linnaeus, 1758)	12.1	73.3	8.9	54.2	44.1	66.7	25.4	64.3
Valvata cristata O. F. Müller, 1774	0.8	6.7	0.1	8.3	0.2	3.3	-	-
Valvata pulchella Studer, 1820	0.7	10.0	4.3	25.0	0.2	6.7	-	-
Valvata piscinalis (O. F. Müller, 1774)	22.4	40.0	27.7	29.2	1.0	20.0	4.6	28.6
Valvata naticina Menke,1845	0.2	3.3	-	-	-	-	0.4	3.6
Acroloxus lacustris (Linnaeus, 1758)	4.5	10.0	-	-	0.2	3.3	1.1	10.7
Lymnaea stagnalis (Linnaeus, 1758)	4.9	60.0	3.0	58.3	5.7	63.3	3.7	60.7
Lymnaea palustris (O. F. Müller, 1774)	0.1	6.7	-	-	0.3	3.3	0.3	10.7
Lymnaea corvus (Gmelin, 1791)	1.4	26.7	7.4	20.8	2.2	40.0	0.8	17.9
Lymnaea truncatula (O. F. Müller, 1774)	0.3	10.0	0.1	4.2	0.4	10.0	-	-
Lymnaea auricularia (Linnaeus, 1758)	-	-	0.1	12.5	0.2	3.3	-	-
Lymnaea peregra (O. F. Müller, 1774)	19.2	80.0	17.9	41.7	4.9	60.0	17.0	78.6
Planorbis planorbis (Linnaeus,1758)	1.5	10.0	3.9	33.3	5.2	33.3	2.2	21.4
Planorbis carinatus O. F. Müller, 1774	0.1	3.3	-	-	-	-	-	-
Anisus spirorbis (Linnaeus, 1758)	1.1	16.7	7.0	45.8	0.4	16.7	-	-
Anisus vortex (Linnaeus, 1758)	6.2	33.3	3.0	45.8	8.6	36.7	3.4	50.0
Bathyomphalus contortus (Linnaeus, 1758)	1.3	16.7	0.6	29.2	2.0	10.0	0.3	3.6
Gyraulus albus (O. F. Müller, 1774)	1.7	30.0	0.2	12.5	4.2	36.7	3.3	25.0
Gyraulus crista (Linnaeus, 1758)	-	-	-	-	0.2	3.3	-	-
Hippeutis complanatus (Linnaeus, 1758)	-	-	-	-	-	-	0.1	3.6
Segmentina nitida (O. F. Müller, 1774)	0.1	3.3	-	-	0.2	10.0	0.2	10.7
Planorbarius corneus (Linnaeus, 1758)	1.7	20.0	3.5	50.0	9.6	73.3	4.4	39.3
Ancylus fluviatilis O. F. Müller, 1774	18.2	30.0	0.1	4.2	-	-	2.5	32.1
Physa fontinalis (Linnaeus, 1758)	0.8	16.7	4.2	75.0	5.6	50.0	4.7	57.1
Aplexa hypnorum (Linnaeus, 1758)	0.4	13.3	1.2	29.2	-	-	-	-
Σ of species	23		19		22		18	
Σ of specimens	2,228		2,794		1,977		2,654	

0.0 to 2.80 and from 0.0 to 0.88, respectively (Fig. 2b). From site 5 to site 8, the values of H' indices showed a decreasing and increasing tendency; at site 5 the value of H' index reached 1.98, at site 6 the value of H' reached 2.71, at site 7 and 8 values reached 1.94 and 2.32, respectively. The Evenness index value increased from site 5, where the structure of gastropod dominance started equalizing. In the Sona River the values of H' and J indices ranged from 0.81 (site 1) to 2.93 (site 10), and 0.43 (site 8) to 0.91 (site 4). The maximum value of H' = 2.93 for the gastropods community occurred at site 10 (Fig. 2c). The values of H' and J indices ranged from 1.4 to 2.83 or 0.61 to 0.71, respectively, in the Wkra River (Fig. 2d).

Analysis of the Spearman Rank Correlation Coefficent r_s

The Spearman Rank Correlation Coefficent r_s (n = 38) showed a lack of statistically significant correlations between gastropod density ($r_s = 0.36$, $P = 0.12$), the number of species ($r_s = 0.06$, $P = 0.80$) and the organic matter content in the bottom sediments. There were statistically significant positive correlations between gastropod density and the number of species ($r_s = 0.65$, $P \leq 0.001$) and magnesium concentration ($r_s = 0.44$, $P \leq 0.05$) in the water, the number of species and dissolved oxygen concentration ($r_s = 0.46$, $P \leq 0.05$) (Table 5). The density of gastropods varied from 10 to 135 individuals/m^2 below 11.0 mg Mg/dm^3,

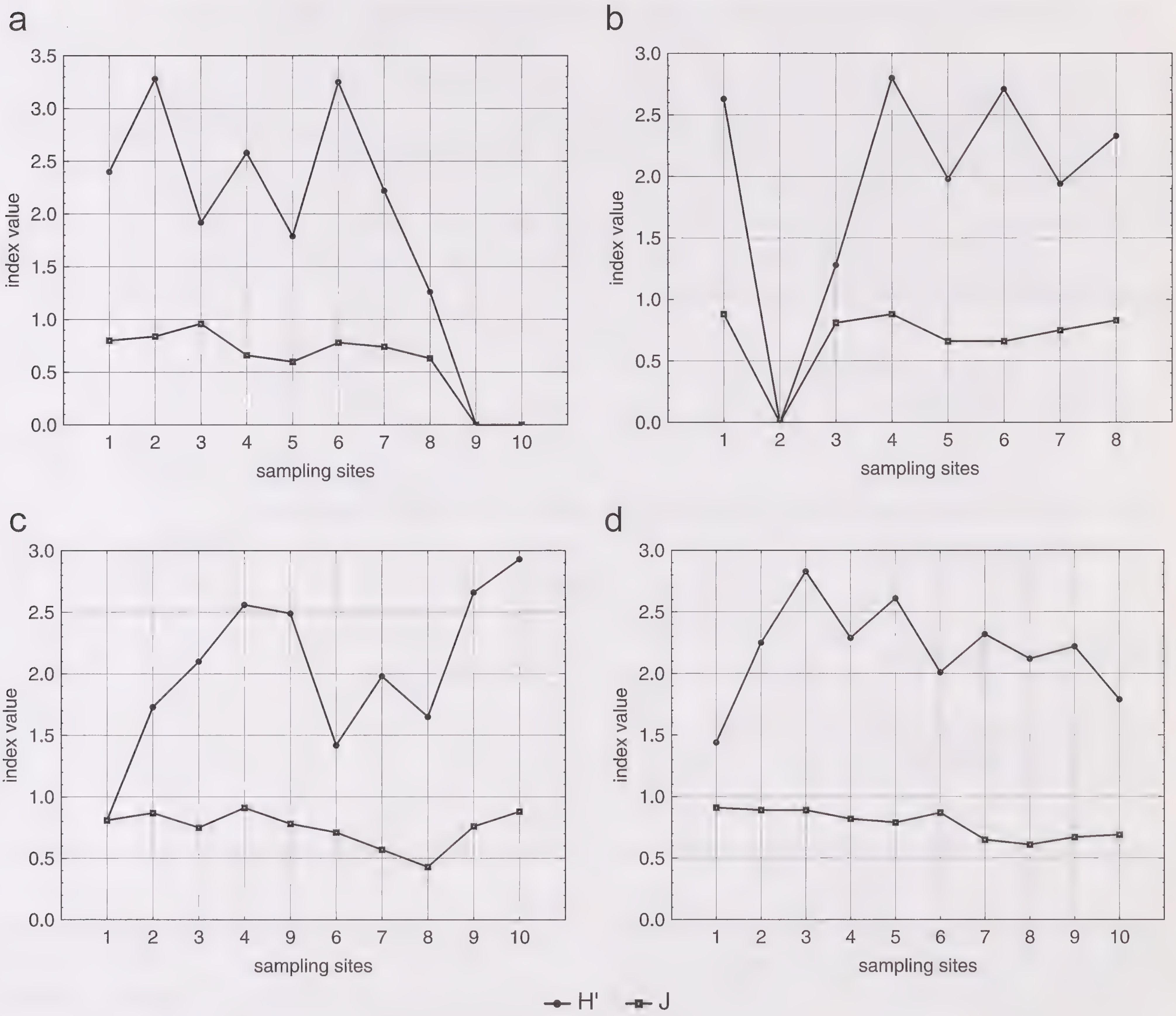

FIG. 2. The value of H' and J for the gastropod communities in the rivers of the Ciechanowska Upland. (a): The value of H' and J for the gastropod communities in the Łydynia River; (b): The value of H' and J for the gastropod communities in the Pełta River; (c): The value of H' and J for the gastropod communities in the Sona River; (d): The value of H' and J for the gastropod communities in the Wkra River.

TABLE 5. The values of the Spearman Rank Correlation Coefficent r_S.

Pair of factors	Value of the Spearman Rank Correlation Coefficent r_s
Specimen density and number of species	0.65 $P \leq 0.001$
Specimen density and magnesium concentration	0.44 $P \leq 0.05$
Number of species and dissolved oxygen concentration	0.46 $P \leq 0.05$
Density of *Viviparus viviparus* and pH	0.51 $P \leq 0.02$
Density of *Viviparus viviparus* and BOD_5	0.51 $P \leq 0.02$
Density of *Viviparus viviparus* and chlorophyll *a* concentration	0.56 $P \leq 0.01$
Density of *Lymnaea peregra* and conductivity	-0.54 $P \leq 0.01$
Density of *Lymnaea peregra* and calcium concentration	-0.47 $P \leq 0.05$
Density of *Lymnaea peregra* and sulphates concentration	-0.47 $P \leq 0.05$

TABLE 6. Gastropod distribution depending on the parameters of the water.

Parameter	Range	Density (individuals/m^2)
Density of *Viviparus viviparus* depending on pH, BOD$_5$ and chlorophyll *a*		
pH	≤ 8.0	0–36
	8.1–8.3	0–70
	≥ 8.4	17–96
BOD$_5$ (mg O$_2$/dm^3)	≤ 3.8	0–38
	3.9–5.0	0–70
	≥ 5.1	21–74
Chlorophyll *a* (µg/dm^3)	≤ 10.0	0–36
	10.1–15.0	3–39
	≥ 15.1	1–96
Density of *Lymnaea peregra* depending on conductivity, calcium and sulphates		
Conductivity (µSxcm^{-1})	≤ 500.0	12–90
	501.0–600.0	0–34
	≥ 601.0	0–18
Calcium (mg Ca/dm^3)	≤ 85.0	1–90
	85.1–95.0	3–61
	≥ 95.1	0–18
Sulphates (mg SO$_4$/dm^3)	≤ 40.0	12–90
	40.1–60.0	1–61
	≥ 60.1	0–13

from 10 to 223 individuals/m^2 at 11.1–17.8 mg Mg/dm^3 and from 36 to 230 individuals/m^2 at above 17.9 mg Mg/dm^3. The number of gastropod species varied from 1 to 9 below 6.6 mg O$_2$/dm^3 in the water, from 3 to 9 at 6.7–7.1 mg O$_2$/dm^3 and from 6 to 10 at above 7.2 mg O$_2$/dm^3. There were statistically significant positive correlations between the density of *Viviparus viviparus* and pH, BOD$_5$, chlorophyll *a*, and a negative correlation between density of *Lymnaea peregra* and any of conductivity, calcium and sulphate concentration (Tables 5, 6).

Fequency Analysis of Gastropod Communities

The result of the frequency analysis showed statistically significant positive (+), negative (-) or lack of associations (0) between certain gastropod species, for example, *Bithynia tentaculata, Lymnaea stagnalis* (Linnaeus, 1758), *Lymnaea peregra* or *Anisus vortex* (Linnaeus, 1758) and the substratum in the Łydynia River ($\chi^2_6 = 64.46$, $P < 0.01$), the Pełta River ($\chi^2_6 = 205.64$, $P < 0.01$), the Sona River ($\chi^2_6 = 62.03$, $P < 0.01$), and the Wkra River ($\chi^2_6 = 175.8$, $P < 0.01$). *Bithynia tentaculata* was positively as-

TABLE 7. Gastropod frequency on the substratum in the rivers of the Ciechanowska Upland.

Species	Łydynia River	Pełta River	Sona River	Wkra River
Bithynia tentaculata	sandy-stony (+)	muddy-clay (-)	sandy-stony (0)	sandy-muddy (-) sandy-stony (+)
Lymnaea stagnalis	sandy (-)	muddy-clay (-)	sandy-stony (0)	muddy (+)
Lymnaea peregra	sandy (+)	muddy-stony (-)	sandy-stony (0)	muddy (+)
Anisus vortex	sandy-stony (+) muddy (-)	muddy-stony (-)	sandy-stony (+)	sandy-muddy (+)

sociated with a sandy-stony substratum in the Łydynia River and in the Wkra River an avoiding muddy-clay or sandy-muddy substratum. In the Wkra River, *Lymnaea stagnalis* was positively associated with a muddy substratum. *Lymnaea peregra* was positively associated with a sandy and muddy substratum, whereas *Anisus vortex* was positively associated with a sandy-stony (Łydynia River) and sandy-muddy substratum (Wkra River) (Table 7).

Principal Component Analysis Results

To explore the studied monitoring data set and to examine the similarities of the samples, a chemometrics technique of the Principal Component Analysis was used. As this data set contains measurements performed within different magnitude ranges, the PCA model was constructed for the centered and standardized data. Unfortunately, the reduction of data dimen-

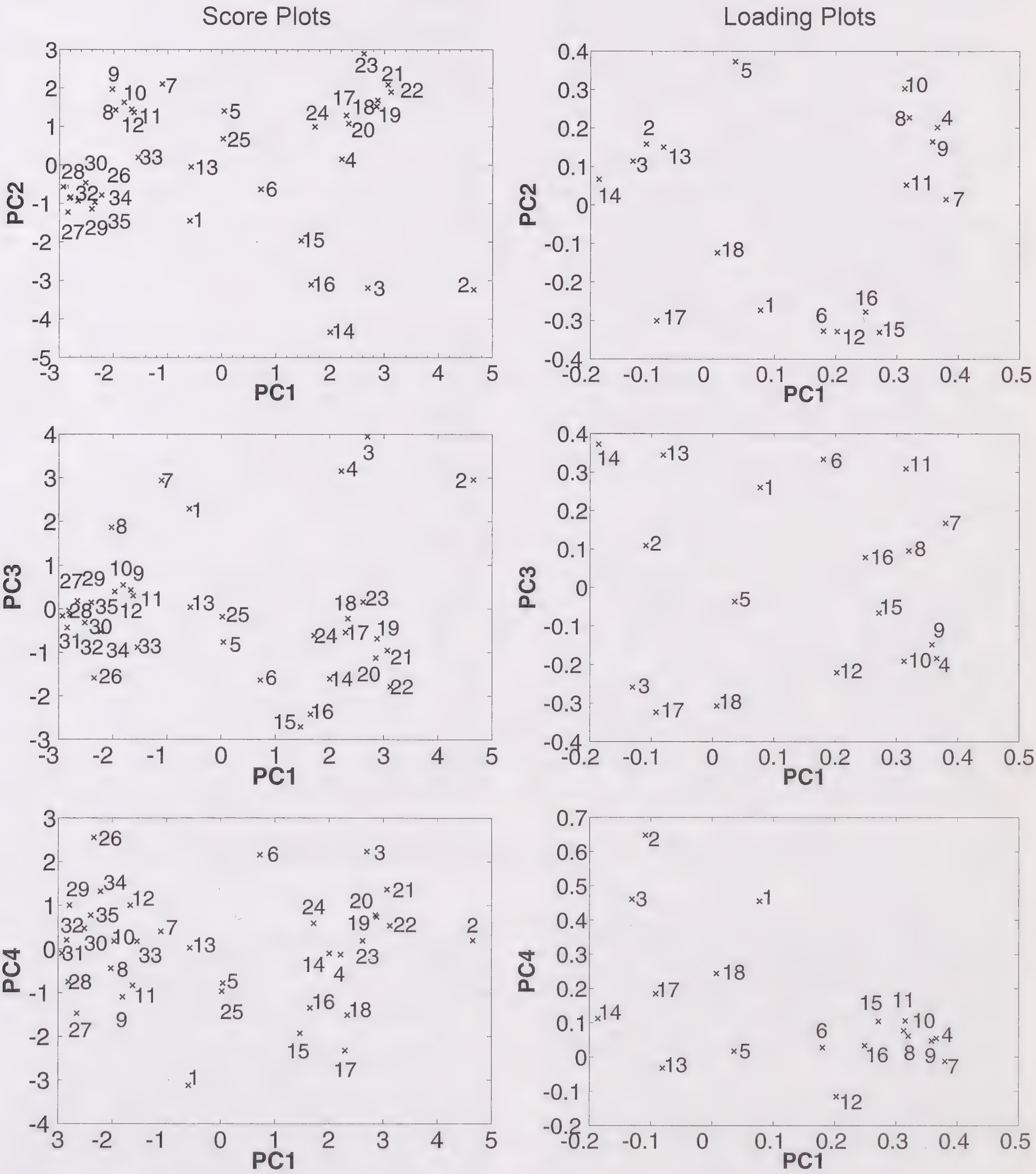

FIG. 3a. Score and loading plots as a result of PCA for centered and standardized data X (35 x 18).

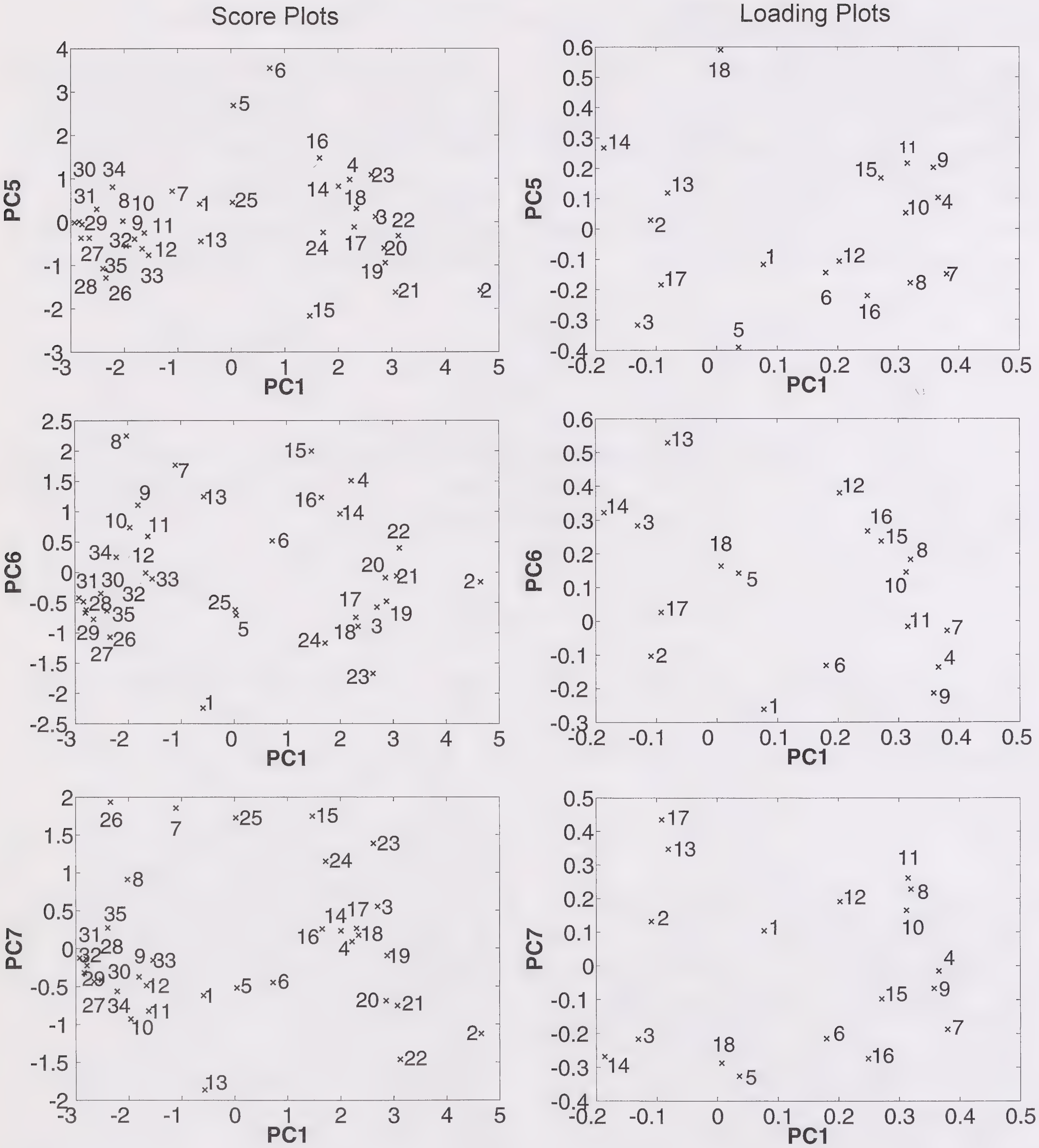

FIG. 3b. Score and loading plots as a result of PCA for centered and standardized data X (35 x 18).

sionality was not too effective, because the PCA model with seven significant Principal Components describes 87.3% of data variance. The score plots and loading plots, which were obtained as a result of this analysis, are presented in Figs. 3a, b.

Based on Figs. 3a, b it is possible to conclude that PC1 reflects the difference between sample sites 8–10 (Sona River), 1–4, 6, 7 (Wkra River), and all remaining samples. Moreover, PC1 reveals the uniqueness of sample site 2 (Pełta River). Based on the loading plot PC1–PC2, it can be stated that sample sites 8–10 (Sona River), 1–4, 6, 7 (Wkra River) differ from the remaining ones, mainly due to the high values of variables 2 (number of species), 3 (pH), 13 (nitrite nitrogen), 17 (chlorophyll *a*) and 14 (nitrate nitrogen). Moreover, PC1 shows the uniqueness of sample site 2 (Pełta River) due to the highest value of parameter 7 (chlorides).

PC2 is constructed mostly due to the difference between sample site 5 (Sona River) and sample sites 2, 3 (Pełta River) 8 and 6 (Łydynia River). Sample site 5 (Sona River) is characterized by a high value of parameter 5 (dissolved oxygen), whereas sample sites 2, 3 (Pełta River) 8 and 6 (Łydynia River) are characterized by the highest value of parameters 6 (BOD_5), 12 (ammonia nitrogen) and 15 (phosphates), as well as the relatively lower values of the remaining parameters.

PC3 shows the difference between sample site 3 (Pełta River) and sample sites 7 and 8 (Łydynia River). Sample site 3 (Pełta River) is characterized by a high value of parameters 1 (gastropod density), 6 (BOD_5), 11 (magnesium), 13 (nitrite nitrogen) and 14 (nitrate nitrogen), whereas sample sites 7 and 8 (Łydynia River) are characterized by the highest value of parameter 17 (chlorophyll *a*).

PC4 reveals the uniqueness of sample site 1 (Pełta River), which is characterized by the lowest value of parameter 2 (number of species), whereas PC5 shows the uniqueness of sample sites 5 and 6 (Pełta River) characterized by the highest value of parameter 18 (density of *Lymnaea peregra*).

Furthermore, PC6 is constructed mainly due to the difference between sample site 8 and sample site 1 (Pełta River), whereas PC7 is constructed due to the difference between sample sites 8 (Sona River) and 5 (Łydynia River). Sample site 8 (Pełta River) is characterized by the highest value of parameter 13 (nitrite nitrogen), whereas sample site 1 (Pełta River) is characterized by the highest value of parameter 1 and 9 (gastropod density and alkalinity). Based on PC7 it is possible to conclude that sample site 8 (Sona River) is characterized by the highest values of parameter 17 (chlorophyll *a*), whereas sample site 5 (Łydynia River) is characterized by the high values of parameters 14 (nitrate nitrogen), 16 (total phosphorus), 18 (density of *Lymnaea peregra*) and 5 (dissolved oxygen).

The PCA loading plots are easy to interpret and reveal a high positive correlation between the parameters, namely:

- cluster 1: parameters 10 and 8 (calcium and sulphates),
- cluster 2: parameters 4 and 9 (conductivity and alkalinity),
- cluster 3: parameters 11 and 7 (magnesium and chlorides),
- cluster 4: parameters 15 and 16 (phosphates and total phosphorus), and
- cluster 5: parameters 6 and 12 (BOD_5 and ammonia nitrogen).

Based on the loading plots it is also possible to observe the negative correlation between parameters 17 and 5 (chlorophyll *a* and dissolved oxygen).

DISCUSSION

Gastropod Communities and their Relation with the Basement Complex and the Substratum

Data of the surveys conducted by Harding et al. (1999) or Dietrich & Anderson (2000) showed that basement complex, substratum, and current velocity influenced the gastropod communities as well as the organic matter content in aquatic ecosystems (Vannote et al., 1980; Delong & Brusven, 1998; Murphy & Giller, 2000).

According to Savage & Pratt (1976), the percentage of organic matter content in the bottom sediments influences the macrophytes richness. In the rivers of the Ciechanowska Upland, the organic matter content in the bottom sediments was low and reached 0.43–28.44%. I did not observe any correlation between gastropod density and either the number of species or the organic matter content of the water. This result is contrary to that of Savage & Gazey (1987), who obtained a statistically significant correlation between *Bithynia tentaculata* occurrence and the content of organic matter. The data from the survey of Dillon (2000), which involved both a substratum and a habitat size, showed an indirect effect of calcium on the gastropod communities. In the New River and its tributaries investigated by Dillon, which passed through a region of gneiss and schist and then through limestone and dolomite, calcium did not affect the abundance of pulmonates directly. The gastropods were rare independent of the calcium content in the water. According to Dillon, the gastropods were also rare in hard water where the tributaries were small. Rosaro & Pietrangelo (1993) claimed that among macroinvertebrates, the gastropods are dominants in streams with a limestone bottom. The result of my survey showed the occurrence mainly of calcite ($CaCO_3$) and dolomite ($CaMg[CO_3]_2$). These minerals could easily dissolve in water. The calcium content in the water varied from 64.90 to 104.70 mg/dm^3. Thus, such concentration of calcium could result in a statistically significant negative correlation between *Lymnaea peregra* density and the calcium content in the water. Collier et al. (1998) came to the conclusion that the gastropod density is negatively correlated with a muddy and a sandy substratum. Strzelec (1993) also showed the negative influence of a muddy substratum on gastropod richness, where only *Lymnaea stagnalis* and *Lymnaea peregra* were present. The results I obtained confirmed their surveys. In the Łydynia River, gastropod richness was poor on a muddy substratum. Besides, I found statistically significant negative association of *Anisus vortex* with a muddy substratum and *Lymnaea stagnalis* on a sandy substratum. But in the Wkra River, *Lymnaea stagnalis* and *Lymnaea peregra* were positively associated with a muddy substratum. *Bithynia tentaculata* in the rivers of the Ciechanowska Upland were associated with a sandy-stony substratum but avoid a muddy-clay and sandy-muddy substratum. This result confirmed Dussart's (1979) results that *Bithynia tentaculata* occurs on the sediment surface and to a lesser degree is associated with macrophytes. The data of surveys done by Chertoprud & Udalov (1996) showed, that in the rivers 19–20 gastropod species occurred on macrophytes, 12–13 species on a substratum consisting of macrophytes and mud and detritus, and 7–10 species on a stony substratum. By contrast, my survey showed, that particular gastropod species were associated with a particular substratum. Stańczykowska (1960a) and Piechocki (1969) found gastropod diversity and abundance were dependent on the specificity of the study area. In the rivers of the southern part of Poland, for example, *Anisus spirorbis* (Linnaeus, 1768), *Acroloxus lacustris* (Linnaeus, 1758), and species of the genus *Valvata* were totally absent (Strzelec, 1993). These species were detected in the rivers of the Ciechanowska Upland. According to Hubendick (1964, 1972), *Acroloxus lacustris* is typical of reservoirs. By contrast, *Acroloxus lacustris* was subdominant in the Łydynia River, subrecedent in the Sona River and recedent in the community in the Wkra River.

Gastropod Communities and their Relation to Physical and Chemical Parameters of Water

According to Strzelec (1993), the number of gastropod species is negatively correlated with the sulphate and magnesium concentration in water if the sulphate concentration level reached above 210.0 mg SO_4/dm^3. I have found that magnesium concentration is positively correlated with gastropod density in the rivers of the Ciechanowska Upland. Gastropod density was highest when magnesium concentration reached a level of 17.9 mg Mg/dm^3. The density of gastropods also decreased when the magnesium concentration decreased. My result is consistent with the investigation of Shieh et al. (1999). They found the highest gastropod density in rivers when the magnesium concentration ranged from 14.55 to 77.27 mg Mg/dm^3. Dillon (2000) showed that the addition of magnesium to water improved the performance of freshwater snails, provided that the concentration of magnesium did not exceed the concentration of calcium. Magnesium may compete for calcium uptake sites. In the rivers of the Ciechanowska Upland, the concentration of magnesium did not exceed the concentration of calcium.

In the rivers of the Ciechanowska Upland, the sulphate concentration influences *Lymnaea peregra* density. The result of my investigation confirmed the survey done by Clenaghan et al. (1998) that showed a positive statistically significant correlation between gastropod density and magnesium concentration as well as pH. They also found a positive correlation between gastropod density and conductivity, density, and calcium concentration, whereas the results of my survey showed a negative correlation.

Dissolved oxygen seems to play a role in gastropod density (Mouthon, 1996). Boycott (1936) and Hubendick (1972) found that *Ancylus fluviatilis* demanded water with a high level of dissolved oxygen. According to Collier et al. (1998), gastropod density is positively correlated with dissolved oxygen. In the rivers of the Ciechanowska Upland, there was a correlation between gastropod density and the concentration of dissolved oxygen. The number of gastropod species varied from 6 to 10 at above 7.2 mg O_2/dm^3 and from 1 to 10 below 7.2 mg O_2/dm^3. The results of my survey are consistent with the opinions of these authors.

Dillon (2000) found that the diversity of freshwater gastropods was at a maximum in waters of about 5–40 mg Ca/dm^3. Dussart (1976) claimed that the occurrence of *Bithynia tentaculata* is limited to hard- and medium-hard water. The calcium content in the rivers of the Ciechanowska Upland varied from 64.90 to 104.70 mg Ca/dm^3 and influenced *Lymnaea peregra* density only. If the calcium concentration increased up to 95.1 mg Ca/dm^3, the density of *Lymnaea peregra* decreased. If the calcium concentration reached 85.0 mg Ca/dm^3, the density of *Lymnaea peregra* increased. Thus, the results of my survey confirmed the fact that the number of specimens per m^2 reaches higher levels in hard water according to Dussart's classification. Boycott (1936) showed that *Lymnaea peregra* was most common in water with a calcium concentration from 1 mg to 8 mg Ca/dm^3. Briers (2003) found that variation in the environmental calcium requirement is an important factor in determining the geographical ranges of freshwater gastropods. According to the environmental requirements, *Lymnaea peregra* is classified as a non-calciphilous species (Boycott, 1936; Briers, 2003). According to McMahon (1983), more than 45% of freshwater basommatophoran snails occur only in relatively hard waters, that is, above 25 mg Ca/dm^3. For example, the optimal value of calcium concentration in water for *Lymnaea palustris* (O. F. Müller, 1774) amounts to 30.0–280.0 mg Ca/dm^3, whereas for *Ferrissia rivularis* (Say, 1817) it is 4.6–67.6 mg Ca/dm^3. Growth rate, survivorship, or fecundity rates all decline with a reduction in calcium concentration below optimal values. In the rivers of the Ciechanowska Upland, calcium concentration from 40.0 to 85.0 mg Ca/dm^3 may play a role in gastropod density. Above this concentration, gastropod density decreased. According to Young (1975), if the level of calcium in water is higher than 5.0 mg Ca/dm^3, other factors determine the abundance and density of gastropods. The negative correlation between *Lymnaea peregra* density and a high calcium content in water may explain the statistically significant association of this species with a muddy substratum. A muddy substratum reduces calcium concentration in water by colloid absorption. Calcium does not accumulate in water until a muddy substratum is saturated with calcium (Dussart, 1976). Thus, a muddy substratum “protects” *Lymnaea peregra* against an excessive calcium concentration in water. Shieh et al. (1999) obtained a negative correlation between alkalinity, conductivity and gastropod richness, as well as a positive one between alkalinity and gastropod density. In the rivers of the Ciechanowska Upland, conductivity negatively influences *Lymnaea peregra* density only. If the conductivity increases above 601.0 µS x cm^{-1}, the density of *Lymnaea peregra* decreases. I have found a statistically significant correlation between conductivity and gastropod density, but not between conductivity and the number of gastropod species.

Jokinen (1985) claimed that the number of the pulmonate species is correlated with alkalinity and the catchment area. This result of the survey is easy to understand, because if the level of alkalinity is high, the rate of the allochtonous organic matter exchange is high compared to the lower value of alkalinity in smaller rivers. Thus, rivers with a higher value of alkalinity can feed more invertebrate species. Clarke & Scruton (1997) claimed that in rivers the absence of gastropods is connected with the pH values of water. This conclusion confirmed an investigation by Herrmann et al. (1993), who noticed a rapid decrease in the number of gastropod species if the pH decreased to 5.5. In the rivers of the Ciechanowska Upland, the density of *Viviparus viviparus* decreased if the pH value decreased. The Ciechanowska Upland is a typical agricultural area. The physical and chemical analysis of the water showed high concentration of nitrate nitrogen, total phosphorus, and phosphates. Soil fertilization and discharge of municipal and industrial sewage affect the level of chlorophyll *a* in the rivers of the Ciechanowska Upland. I have obtained a statistically significant correlation between *Viviparus viviparus* density and the chlorophyll *a* level. There is no correlation between the density of *Viviparus viviparus* and either nitrate nitrogen or phosphates. However, the level of chlorophyll *a* in water is dependent on the concentration of these nutrients. Thus, *Viviparus viviparus* may play the role of a bioindicator of eutrophication in running waters.

Gastropod Communities and their Relation to Macrophytes

Some gastropod species are associated with bottom sediments as well as with macrophytes, for example, *Bithynia tentaculata* (Kornijów, 1989). According to Soszka (1975) *Bithynia tentaculata* and *Valvata piscinalis* occur on bottom sediments mainly, and to a lesser degree on macrophytes. *Anisus vortex* is associated mainly with bottom sediments (Kornijów et al., 1990).

In the rivers of the Ciechanowska Upland, gastropods were frequently observed on macrophytes. In the Łydynia River, at one sampling site, *Ancylus fluviatilis* occurred exclusively on *Nuphar lutea* (L.) Sibth. & Sm. *Planorbis carinatus* O. F. Müller, 1774, a species typical of lakes, was present on *Potamogeton crispus* L. Stańczykowska (1960b) and Dvořák & Best (1982) suggested that *Bithynia tentaculata, Lymnaea stagnalis, Lymnaea peregra*, and *Anisus vortex* or *Gyraulus albus* (O. F. Müller, 1774) were not typical phytophilous species. I found that these species occurred on macrophyte surfaces as well as on bottom sediments. This result confirmed the survey of Kornijów & Gulati (1992). Brown (1997) claimed that gastropods prefer wider the leaf blades of macrophytes rather than narrow leaf blades because they provide more periphyton. According to Brönmark (1989), *Ancylus fluviatilis* occurs on the lateral surfaces of stones because they are richer in diatoms. *Valvata piscinalis* feeds mainly on detritus, whereas green algae and diatoms constitute only a small part of their diet (Kornijów, 1996). McMahon (1983) showed that bottom sediments play a role for freshwater gastropods because minerals comprise a large component of the periphyton on which many gastropods graze. Calcium in the shells derives both from ingested food and bottom sediments. Thomas & Kowalczyk (1997) claimed that the relationship between macrophytes, periphyton, and gastropods is based on mutualism, whereas Jones et al. (2000) obtained the opposite result. According to Dillon (2003), the effects of calcium, alkalinity, conductivity or pH on gastropod communities may be indirect. Thus, higher values of these parameters may influence the production of food on which gastropods might feed. Kołodziejczyk (1984) found that *Bithynia tentaculata* and *Anisus spirorbis* prefer detritus. Thus, it is likely, that the statistically significant association of those species with the bottom sediments in the rivers of the Ciechanowska Upland may be explained by alimentation relationships.

Gastropods, Macrophytes, and their Bioindicative Values

Many authors consider gastropods as a good indicator of sewage, heavy metals or radionuclide concentration in rivers (Dregolskaya, 1993; Abdallah et al., 1999; Flessas et al., 2000). Shieh et al. (1999) claimed that gastropods could be indicators of water hardness. Gastropods are more sensitive to pH changes than fish (Økland, 1983). Calcium is not easily accessible to gastropods at a low pH value (Clarke & Scruton, 1997). Thus, gastropods are good indicators of the acidification of water environments. The results of my survey show that certain physical and chemical parameters influenced the optimal gastropod population development. According to Mouthon (1996), *Valvata piscinalis* and *Viviparus viviparus* showed an association with aquatic environments that are abundant in phosphates and nitrates. In the rivers of the Ciechanowska Upland, the correlation between *Viviparus viviparus* density and the BOD_5 level was statistically significant, so that a moderate load of organic matter appeared to promote its occurrence. *Valvata piscinalis* and *Viviparus viviparus* were numerous in the middle and lower course of rivers, where the concentration of phosphates and total phosphorus was high. In the rivers of the Ciechanowska Upland, gastropods could also be indicators of the concentration of dissolved oxygen. The number of gastropod species increased with an increasing level of dissolved oxygen in water. *Viviparus viviparus* and *Lymnaea peregra* could be indicators of anthropopressure. *Viviparus viviparus* density increased when pH, BOD_5 and the level of nutrients also increased.

According to Thiébaut & Muller (1999), the occurrence of *Elodea canadensis* Michx. in the middle course indicates a progression of eutrophication, whereas *Berula erecta* (Huds.) Coville or *Lemna trisulca* L. indicates oligotrophic water. In the case of my survey, *Elodea canadensis* occurred in the middle course of the rivers, but *Berula erecta* occurred in the upper course of the Pełta River only.

Diversity Indices

According to Trojan (2000), gastropod community diversity depends on the size of the study area, basement complex, and anthropopressure. Clausen & Biggs (1997) showed that the values of the Shannon-Wiener index are high for invertebrate communities and among these for gastropod communities that occurred in smaller rivers compared to communites in larger ones. I have obtained a similar result for the gastropod communities which occur in the Łydynia River and the Sona River, which are tributaries of the Wkra River. Chedwick et al. (1986) found that the Shannon-Wiener index values decreased in rivers under the influence of the metal and mining industries. By contrast, García-Criado et al. (1999) claimed that the Shannon-Wiener index does not reflect the environmental impact of coal mines on community structure in rivers. Perhaps, if they had carried out their survey at genus level instead of the family level, the environmental influences would have been more distinctive. In my survey, the effects on physical and chemical parameters differed from effects caused by the mining industry. The values of H’ and J indices were high for gastropod communities in the headwater of the Łydynia River only. In the lower course of the

Łydynia River, the values decrease to 0. A similar result was obtained by Shieh et al. (1999) in rivers under the influence of industry and agriculture. Strzelec et al. (1999) also claimed that along rivers of the Śląska Upland (southern Poland) gastropod communities decrease. I have not observed a similar tendency in the Sona, Pełta, and Wkra rivers. In the rivers of the Ciechanowska Upland, diversity indices varied from site to site, but did not decrease along the river pollution gradient. The similar tendency was observed by Cao et al. (1996) in the River Trent system: diversity indices varied widely between sites, but did not show a consistent decrease with increasing pollution levels; low values were recorded at both clear and polluted sites.

CONCLUSIONS

Gastropod communities in the rivers of the Ciechanowska Upland are influenced by bottom sediments, the physical and chemical parameters of water, and macrophyte abundance, which are mainly typical of eutrophic water. *Valvata naticina* Menke, 1845, threatened with extinction in Poland, is present in the Łydynia River and in the Wkra River. The result of the survey showed that the range of certain physical and chemical parameters influenced the development of optimal gastropod population and the threshold values at which inhibition of their development occurred. In the rivers of the Ciechanowska Upland, gastropods can be biological indicators of the dissolved oxygen level in water. When the level of dissolved oxygen increased, the number of gastropods species also increased. *Viviparus viviparus* and *Lymnaea peregra* can be indicators of anthropopressure. *Viviparus viviparus* density increased with increases in pH, BOD_5, and the concentration of chlorophyll *a*. *Lymnaea peregra* density decreased if calcium, sulphates, and conductivity increased. The values of diversity indices varied from site to site, but did not decrease along the river pollution gradient, so the result I have obtained is not easy to interpret. Perhaps the diversity indices do not reflect the anthropopressure degree in the rivers of the Ciechanowska Upland. Diversity indices may be more useful for aquatic ecosystems that are more polluted than the rivers of the Ciechanowska Upland. The PCA loading plots mainly showed a high correlation between the physical and chemical parameters.

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FOSSIL LAND SNAIL FAUNAS OF PORTO SANTO, MADEIRAN ARCHIPELAGO: CHANGE AND STASIS IN PLEISTOCENE TO RECENT TIMES

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ABSTRACT

Samples of fossil land snails were made at 14 sites on the island of Porto Santo, Madeiran archipelago. Material in stratigraphical sequences could be dated by A/I racemization calibrated against radiocarbon dating, and against Uranium/Thorium estimates based on Madeiran material. Although errors associated with the oldest samples are large, it is clear that the oldest shell-bearing deposits are at least 300 ka old, and probably much older. Some taxonomic difficulties have been resolved by morphometric studies. The geographical pattern in the fossils (in both faunal composition and morphometrics) resembles closely that seen today, the southwest of the island being particularly distinctive. Of 58 native taxa found in the deposits, 22 are extinct on the island, though four still survive on Madeira. Extinction has been greater in the southwest than elsewhere, and early fossil faunas there are richer than later ones. As on Madeira, more than half of this extinction seems likely to be the consequence of human disturbance, which has also reduced and fragmented the ranges of other species. The overall pattern is of distributional stasis in many species, reduction of range or extinction in many others, but very few cases of range expansion. Neither extinctions nor changes in apparent abundance can be related to known changes in global climate over the period involved. This relative stasis is in marked contrast to the situation on Madeira, only 40 km away, where there are temporal shifts in the fauna, and evidence of colonization events. Possible reasons for this difference are discussed.

Key words: Fossil land snails, Porto Santo, Pleistocene, Holocene.

INTRODUCTION

The Madeiran archipelago has a rich land snail fauna, with many endemic species and high levels of differentiation between habitats and islands. This diversity has been studied since the 19th century (Lowe, 1831, 1852, 1854; Albers, 1854; see Wilson, 1970). Some of the features have been reviewed, along with new data, by Waldén (1983, 1984), by Cameron & Cook (2001) for Madeira, Cameron et al. (1996b) and Seddon (1995) for Porto Santo, and Cameron & Cook (1999) for the Deserta islands. In addition to information on modern distribution and species associations, there are records from fossil deposits on the history of the fauna, making the islands an exceptional centre for study of an evolutionary radiation. We have examined dated fossil material from the eastern peninsula of Madeira (Cook et al., 1993; Goodfriend, 1994, 1996) and from the Desertas (Cameron & Cook, 1999).

The fossil faunas of Porto Santo have received less attention. Wollaston (1878) reviewed early work, and there have been some later taxonomic and individual site reports. This paper gives results of extensive sampling on Porto Santo and its adjacent islets by Goodfriend, Cameron and Cook, and, independently, by Seddon. Both series are accompanied by stratigraphical notes, but only the former was subject to dating analysis, carried out by Goodfriend, and incomplete at the time of his death. The extent of shell-bearing de-

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*Authorship of this paper is in alphabetical order. The collections were made by Goodfriend, aided by Cameron and Cook, and at different times and places, by Seddon. The dating is entirely the work of Glenn Goodfriend, who died in 2002; had he survived he would have been senior author. As it is, the paper is dedicated to him in memory of his companionship and pioneering work on geochronology.

posits on the island is vast (at least half the present surface area is covered by aeolian or colluvial deposits) and the stratigraphy is complex. Sampling sites were spread broadly across the island, providing an opportunity to look not only at species associations and possible temporal changes but also at microgeographical variation. There are marked differences in the present fauna from one part of the island to another (Cameron et al., 1996b) and some interesting evolutionary patterns.

METHODS

Goodfriend's Samples

(a) *Stratigraphy and Dating.* Locations were sought where exposed sequences of aeolian sands or colluvial deposits could be examined for molluscan shells (Fig. 1). Stratigraphical notes were made in the field. Snail shell samples (and in a few cases bird egg shell) that were definitely *in situ* were collected for dating. Chronology is based on measurements of D-alloisoleucine: L-isoleucine (A/I) ratio (Goodfriend, 1987) using gas chromatography (GC) and high performance liquid chromatography (HPLC), calibrated against radiocarbon dating. The snail species tested were *Caseolus bowdichianus*, *C. punctulatus*, *Helix subplicatus*, *Leptaxis chrysomela*, and *Theba pisana*, chosen because their shells are relatively robust and they cover a wide range of possible dates. *Theba pisana* is a non-endemic that arrived recently, whereas the others are endemic to the islands.

(b) *Site Choice and Methods.* Goodfriend took samples at 13 sites in 1990 and 1993, and an additional site (No. 11) was sampled by Cook in 1993 (Fig. 1). At five sites (1, 2, 6, 7 and 12/10), several samples were taken to cover the apparent stratigraphical range. At Fonte de Areia (Sites 1 and S3), the exposed sections were approximately 1 km long and up to 40 m

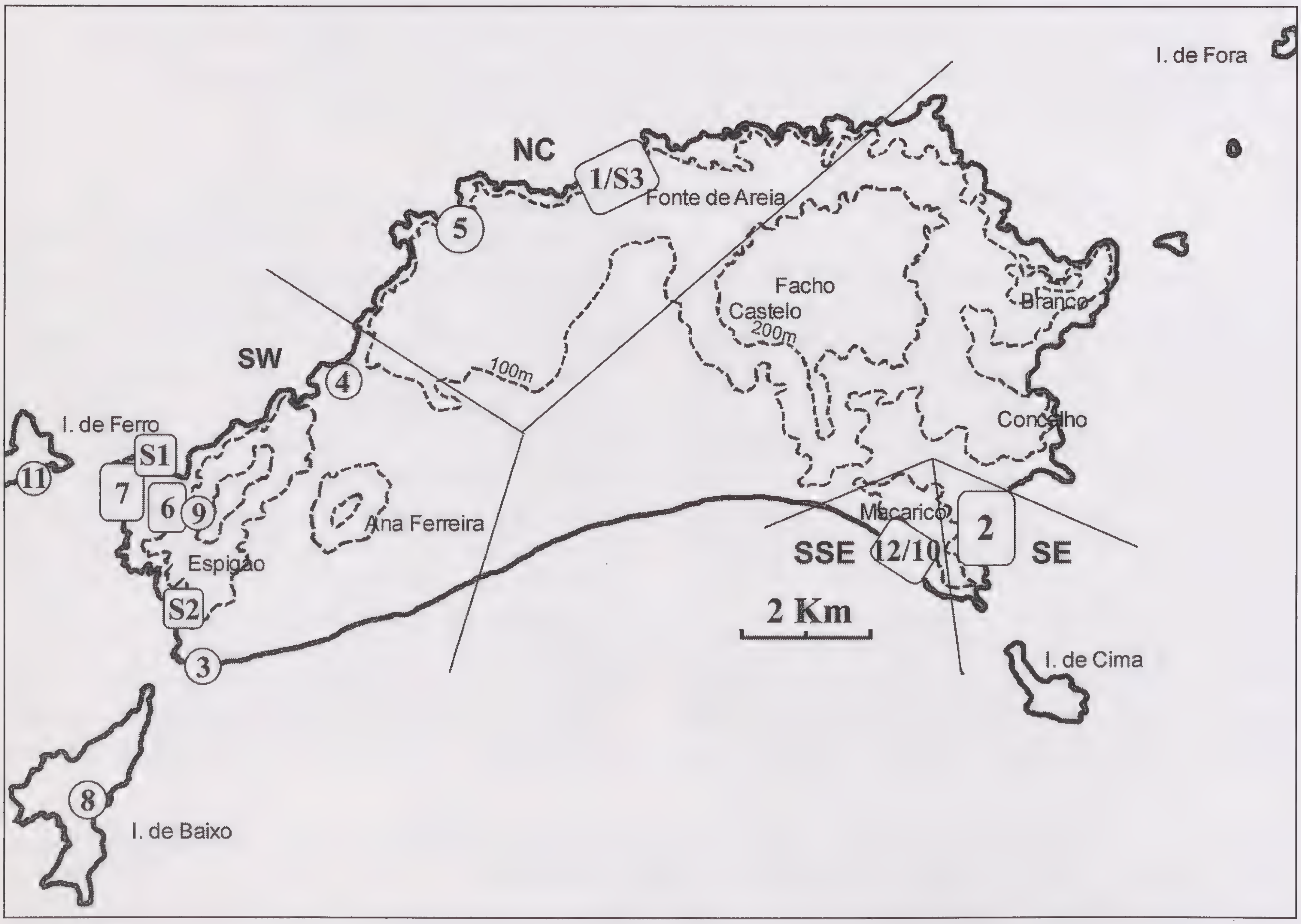

FIG. 1. Map of Porto Santo and islets showing collecting sites. Site13 is within the area of site10/12. Circles:sites with only one or two samples only; squares: sites with three or more samples. Site numbers prefixed by 'S' are Seddon's sites. Regions: SW: southwest, NC: north-central, SE: southeast, SSE: south-southeast.

in height, and although sampling was not possible from a single vertical column, the stratigraphical position of each sample could be reliably determined. The nature of the terrain elsewhere was often such that collecting had to be distributed amongst several exposures. At most, these were separated by 500 m; generally, the distance was less than 100 m. Sites 10 and 12 were adjacent and have been combined as a single site. Relative stratigraphical positions could be determined by a combination of the character of strata, topography and A/I values. Other sites were sampled in a more exploratory way, looking at one or two strata only. Shells of terrestrial snails were collected (see below) at all but Site 13, within the area of 12/10, from which fossil limpet shells were removed for A/I analysis to provide a basal date for sediments sampled in Sites 10 and 12. At some sites with few shells material was collected solely for A/I analysis.

Shells were collected from cleaned and vertically defined sections of the profile, and character and depth of exposed sediments were recorded. In many cases, the matrix was indurated and shells were dug out singly. Sometimes it was possible to sieve a section of the profile through meshes down to 0.5 mm. Since extraction techniques varied, our analyses generally concern presence and absence data, but the numbers of shells of each species found are shown in the appendices and are discussed in particular contexts.

(c) *Sites.* Goodfriend made stratigraphical, topographical and location notes for each site and sample. From these, and from the results of A/I analyses, we have ordered samples by age, using the stratigraphy to place those lacking A/I determinations. Cases where stratigraphical position and A/I ratios give contradictory results are explained in the appendix. Site descriptions are provided in Appendix 1. The locations with most complete samples are Sites 1, 2, 6, 7, 12/10, S1, S2, and S3, and some analyses use only these series.

Seddon's Samples

Seddon's samples were taken at 13 sites, usually from cleaned and vertically defined sections. Most were of fixed substrate weight, and the shells were extracted by sieving down to 0.5 mm mesh. Many were post-human colonisation deposits, containing the introduced *Theba pisana.* Here we consider only three sites with older strata, where the stratigraphical context of each sample was clear. Locations of Seddon's samples are shown in Fig. 1, with details in Appendix 1.

IDENTIFICATION AND NOMENCLATURE

Most of the taxa known from fossil deposits on Porto Santo were described in the nineteenth century, and discussed by Wollaston (1878). The fauna of the archipelago as a whole was revised by Waldén (1983), including all taxa known only from fossil shells, and incorporating work done subsequent to Wollaston's monograph. Waldén visited many museums, comparing types with other material in the museums, and with his own collections. In the Natural History Museum, London, in the National Museum of Wales, Cardiff, and in the Manchester Museum, he inserted labels giving his diagnoses of lots. We have compared our material with his diagnoses.

Waldén (1983) used trinomials (nominal subspecies) for forms with distinctive features that did not, in his view, merit species status. It is not always clear whether these forms have separate geographical or temporal ranges. Many were regarded as varieties by Wollaston (1878), whose material was usually not localised within islands.

Of necessity, fossil taxa are defined on conchological characters, despite their known unreliability in living taxa. Erosion of surface features can make determination difficult, especially in the genus *Caseolus.*

Since Waldén's (1983) account, some new taxa have been described, the nomenclature reassessed and a new provisional checklist published (Bank et al., 2002). Seddon (in press) will provide an up-to-date synonymy, and papers describing new taxa are included in the references. We have followed the names and sequence in Waldén (1983), with some exceptions, additions and qualifications. We have refrained from introducing new names, but use a few synonymies and aggregate taxa where characters are hard to determine. A wider spread of samples often blurs the distinction between taxa described from single samples in which apparently distinctive forms were found. Goodfriend's material was identified by RADC, and Seddon's material by her. Subsequently, the two sets were compared for consistency. Exceptions are:

- *Leiostyla subcorneocostata* Seddon, 1990. A close relative of the extant *L. corneocostata* (Wollaston, 1878).
- *Leiostyla espigaoensis* Seddon, 1990.
- *Phenacolimax portosanctanus* Groh & Hemmen, 1986a.
- *Phenacolimax crassus* Groh & Hemmen, 1986a.
- *Eucobresia media* (Lowe, 1854). Omitted by Waldén (1983) but reinstated by Groh & Hemmen (1986a).
- *Heterostoma paupercula* (Lowe, 1831). Includes all forms referred by Waldén (1983) to *Heterostoma* or *Steenbergia.* A morphometric analysis is given below.
- *Geomitra acarinata* Hemmen & Groh, 1985.
- *Geomitra gerberi* Groh & Hemmen, 1986b.
- *Spirorbula latinea* (Paiva, 1866). This name is not used, based on morphometric evidence (see below) that it is an extreme form of *S. depauperata* (Lowe, 1831).
- *Caseolus compactus* (Lowe, 1831). Forms described under this name undoubtedly belong to more than one species. Waldén (1983) listed two extant, and three extinct subspecies, relating some to varieties described by Wollaston (1878). The diagnostic characters often involve shell sculpture characters, which are frequently abraded in fossil shells, and, sometimes seem to show continuous variation. We have generally distinguished between large and small shells, as the distinction is often marked, with no evident intermediates. In the best-preserved material, it is sometimes possible to assign shells to the designated subspecies *C. c. ariensis* Waldén, 1983, and *C. c. vigiae* Waldén, 1983. In the latter case, comparison with identified material in museums suggests that there may be confusion with *C. baixoensis* Waldén, 1983, a species allied to *C. commixtus* (Lowe, 1854) (see below). We have not recognised the latter in our samples.
- *Caseolus consors* (Lowe, 1831). In general, this is a well-characterised species, but in older deposits it sometimes resembles large forms of *C. compactus*.
- *Caseolus abjectus* (Lowe, 1831). In the modern fauna, this species has a close relative, *Caseolus commixtus* (Lowe, 1854), which is usually clearly distinguishable. In subfossil samples, the distinction is hard to make: the taxon *C. abjectus* agg. includes forms that could be placed in *C. commixtus.*
- *Caseolus subcalliferus subcalliferus* (Reeve, 1854), and *C. s. submajor* Waldén, 1983. The latter is very abundant in some Pleistocene/Holocene deposits, but appears to be extinct. The two forms are occasionally found together. We have treated them as species, but have retained Waldén's nomenclature.
- *Caseolus punctulatus* (G. B. Sowerby I, 1824). We include specimens that might be assigned to *C. solidus* (Lowe, 1831). Both species are deemed to be extant, but modern populations show continuous variation in diagnostic characters.
- *Discula bicarinata* (G. B. Sowerby I, 1824). Appears to be distinct at species level from *D. b. aucta* (Wollaston, 1878). We have retained Waldén's nomenclature.
- *Discula oxytropis* (Lowe, 1831). Waldén listed two extinct subspecies, *D. o. subcarinulata* (Wollaston, 1878), and *D. o. vermetiformis* (Lowe, 1854). These taxa are problematic. We present morphometric data below, and treat all our material as *D. oxytropis.*
- *Discula calcigena* (Lowe, 1831), *D. pulvinata* (Lowe, 1831), and *D. attrita* (Lowe, 1831). We have retained these names, despite difficulties with some specimens. Waldén (1983) listed five subspecies of *D. calcigena,* one of which, *D. calcigena discina* is regarded by some as a species. We have not used these subspecific distinctions. In the deposits, and in modern faunas (Cameron et al., 1996b), we find pairs of the three species level taxa present syntopically with no intermediates.
- *Discula albersi* (Lowe, 1852). Regarded as a synonym of *D. bulweri* (Wood, 1828).
- *Leptaxis* sp. In two sites, we have specimens of a form similar to *L. erubescens* (Lowe, 1831) or *L. furva* (Lowe, 1831) but not matching either exactly. *L. furva* is a montane species, known only on Madeira (Wollaston, 1878). *Leptaxis erubescens* occurs in the modern Porto Santan fauna only on the upper slopes of the highest hill, Pico do Facho.
- *Leptaxis* cf. *fluctuosa.* Waldén (1983) regarded this form as a subspecies of *L. chrysomela* (Pfeiffer, 1848). While it is very variable, we regard it as a species-level taxon.
- *Leptaxis granulosa,* Groh, 1983. Known only subfossil (Groh, 1983) and here regarded as a synonym of the also extinct *L. psammophora*.

All species in our samples, with the exception of *Theba pisana* (Müller, 1774), *Punctum pygmaeum* (Draparnaud, 1805), and *Plagyrona placida* (Shuttleworth, 1852), are endemic to the Madeiran archipelago, but *Heterostoma paupercula* and *Leptaxis erubescens*, the latter not recorded in our samples but extant on Porto Santo, have been introduced in the Azores (Backhuys, 1975).

RESULTS

Chronology

A total of 220 A/I racemization determinations was made on material from 51 stratigraphical sections at 12 locations. About three quarters were HPLC measurements and the rest GC; there is good agreement between the two. Excluding three measurements that obviously refer to redeposited shells (in samples 2-6, 2-10 and 7-1) and a section for which there is a single measurement, the variation between sections is highly significant (F = 47.3, 50/165 d.f.). The within-section standard error is 0.0056. The measurements therefore show consistency within sections and allow clear distinctions to be made between them.

Correction and calibration of the A/I data for Porto Santo is incomplete, but at selected levels in the younger parts of sequences radiocarbon analysis of shell carbonate has provided points corresponding approximately to known ages. There are four samples available, ranging from 0.140, corresponding to an age of 3.7 ka, to 0.381, corresponding to > 47 ka. These figures fit well with the 17 records for Madeira (Goodfriend et al., 1996; Cameron & Cook, 1999), which include two Uranium/Thorium dates for older samples. Techniques and qualifications relating to the Madeiran deposits were discussed by Goodfriend et al. (1996). The pooled data from reliable precolonisation determinations for both islands provide a good linear relation of log years on ratio ($\log_n$ years = 10.4822 A/I + 7.2432, r^2 = 0.929). This does not represent the relationship for very recent times (see discussion in Goodfriend et al., 1996), but for intermediate values a ratio of 0.2 corresponds to an age of about 11 ka and 0.4 to about 93 ka. At the limit of available calibration, an A/I ratio of 0.5 corresponds to about 263 ka. At the largest mean A/I ratios (around 0.8) small errors have large effects; the logarithmic equation above gives an estimate of approximately 6 Ma. This is unlikely to be correct, on stratigraphical grounds at least, but the oldest samples may be more than one million years old.

Mean A/I ratios for the 5 GAG sites subject to detailed faunal analysis are shown in Figure 2. Sites 2, 6, and 12 contain no horizons as old as those in Sites 1 and 7. Samples from the base of the uppermost stratigraphical unit of Site 1 (1-1) provide 4 A/I ratios with a range from 0.308 to 0.422 (mean 0.349), and a radiocarbon age of > 47 ka. This is beyond the

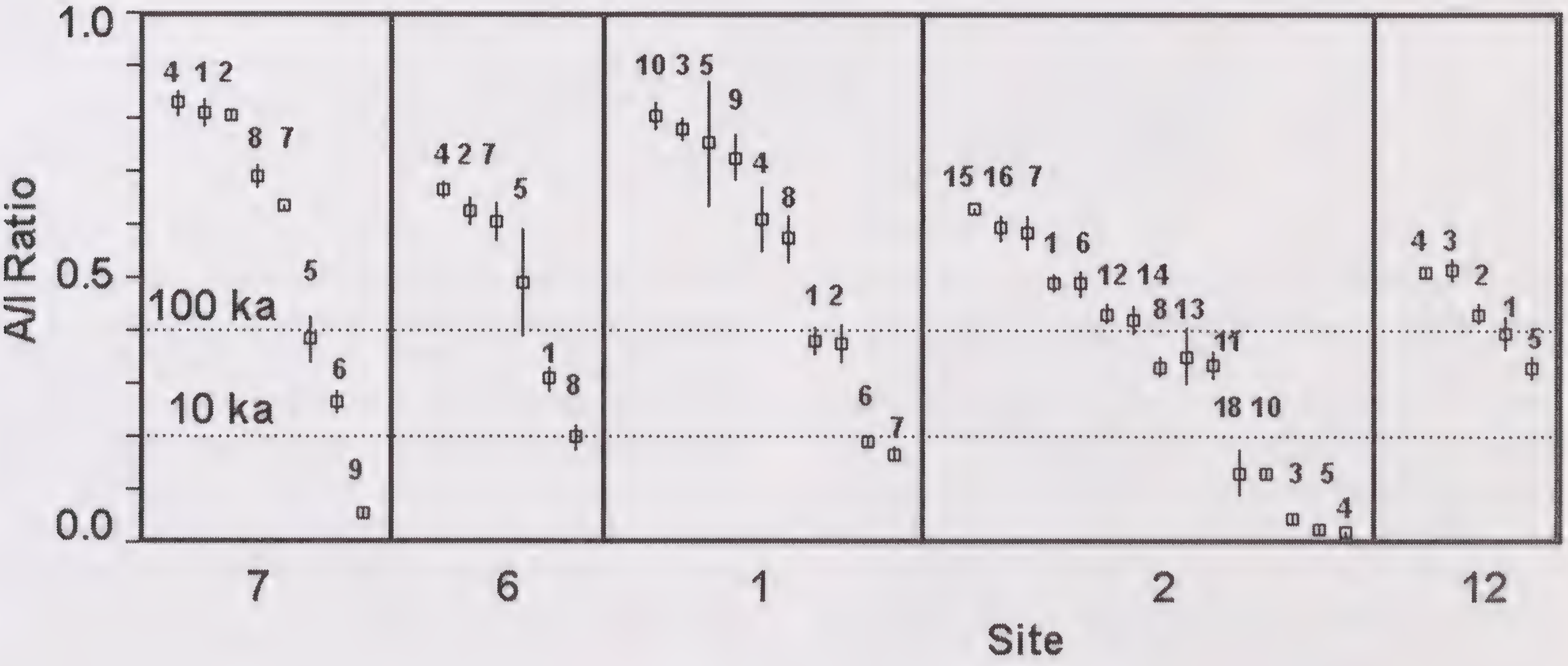

FIG. 2. D-alloisoleucine:L-isoleucine (A/I) values for sampled sections at faunal analysis sites arranged in descending order of age. Means and standard errors are given for readings from each section, and section reference numbers are shown. Dotted lines indicate ages of approximately 10 ka and 100 ka based on calibration by radiocarbon dating.

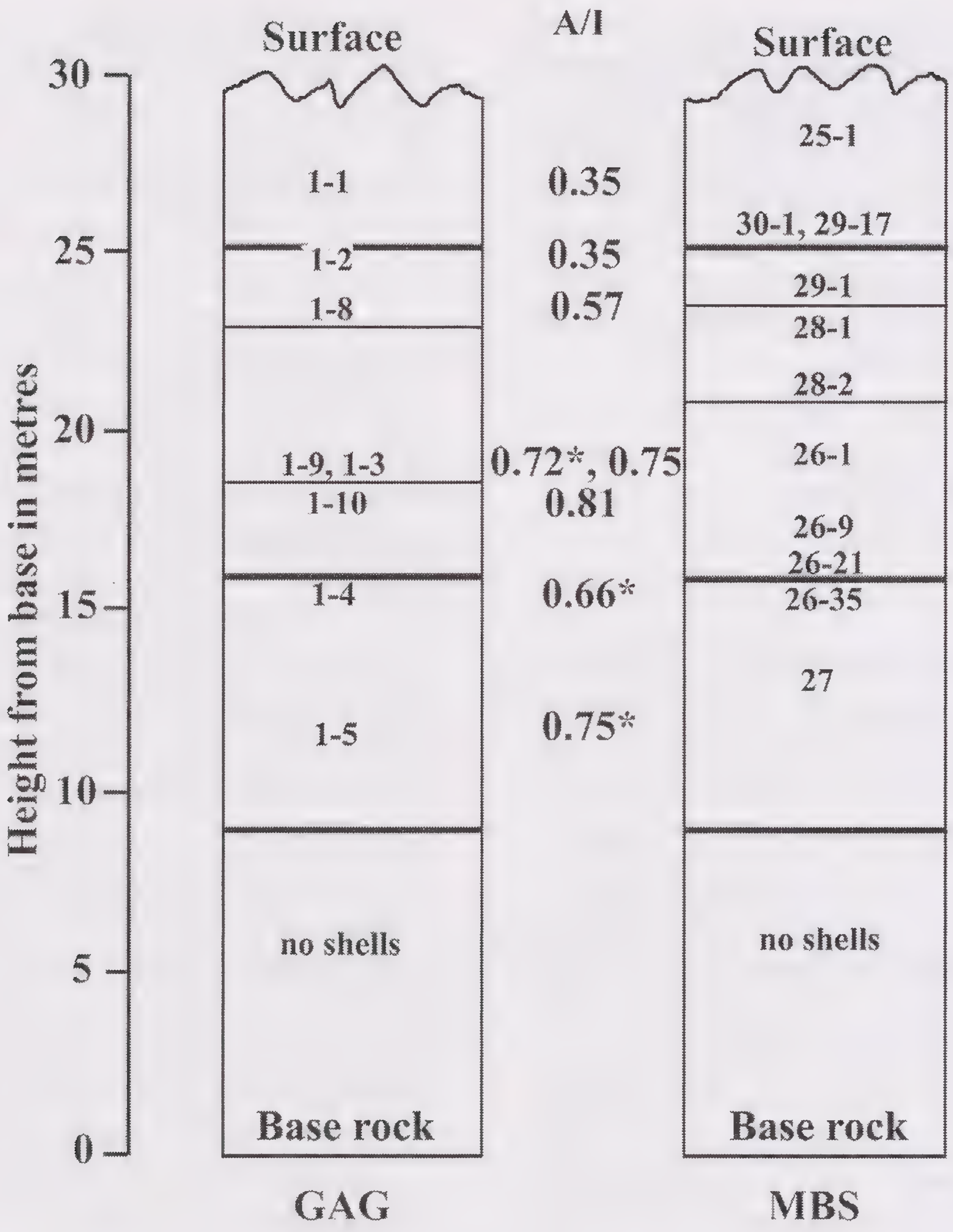

FIG. 3. Diagrammatic stratigraphical section at Fonte da Areia, showing the relative positions of samples from Sites 1 and S3. The sample sequences are shown relative to the base rock underlying the sediments. Thick horizontal lines represent strata boundaries that can be aligned between the two sequences with a high degree of confidence; the thinner lines represent discontinuities in each, which may not be equivalent (see Appendix 1). Mean A/I ratios are shown for Site 1 samples; asterisked values indicate variability in results for individual shells. Note that Site 1 samples do not come from a single column, and the depths of strata vary from place to place in the exposure.

limit of reliable determination, but considerably older than the 21 ka suggested by Lietz & Schwarzbach (1971) for the same horizon.

Fig. 3 compares and aligns samples from Site 1 and Site S3, which come from the same stratigraphical sequence on the north coast, and relates them to the range of A/I values (Appendix 1). Samples 1-6 and 1-7 (Holocene) were made away from the main stratigraphical sequence, and are excluded. Sample S3-27 matches 1-5 in the abundance and composition of its material (Appendices 4 and 7). With the exception of 1-4, all samples less than 20 m from the base have A/I ratios > 0.7, and those between 20 and 24 metres have A/I ratios in the range 0.4–0.7. We are unable to assign the neighboring samples S1 and S2 to any of these bands. S1 has a relatively poor fauna resembling that of Site 6. S2, however, is the most distinctive in the whole array, with seven species unique to it (Appendix 8), of which six are extinct. These deposits are probably old, and their colluvial nature may have resulted in faunal mixing.

Faunal Composition

The faunal composition of successive layers for Sites 7, 6, 1, 2, 12/10, S1, S2 and S3 (sites with more than two samples) is given in

Appendices 2–8. Appendix 9 gives the results for the remaining sites. Overall, 59 taxa were recognised, 22 of which are extinct (37.3%). Given the uncertainties and aggregate categories referred to above, this is a minimum number. The following list 1 shows the status of these taxa, along with that of other taxa recorded on Porto Santo, but not present in these samples (Seddon, in press).

Present, extinct, endemic:
- *Craspedopoma mucronatum*
- *Truncatellina linearis*
- *Staurodon saxicola*
- *Leiostyla subcorneocostata*
- *Leiostyla espigaoensis*
- *Phenacolimax crassus*
- *Phenacolimax portosanctanus*
- *Geomitra acarinata*
- *Caseolus* cf. *compactus vigiae*
- *Caseolus subcalliferus submajor*
- *Caseolus bowdichianus*
- *Actinella arcinella papillosa*
- *Actinella crassiuscula*
- *Actinella morenensis*
- *Discula bicarinata aucta.*
- *Discula cockerelli*
- *Pseudocampylaea lowei*
- *Leptaxis* sp.
- *Leptaxis chrysomela*
- *Leptaxis* cf. *fluctuosa*

Present, extinct, non-endemic:
- *Punctum pygmaeum*
- *Plagyrona placida*

Absent, extinct, endemic:
- *Geomitra gerberi*
- *Discula echinoderma*
- *Leptaxis psammophora*

Present, extant, endemic:
- *Leiostyla calathiscus*
- *Leiostyla monticola*
- *Amphorella melampoides*
- *Amphorella triticea*
- *Amphorella oryza*
- *Amphorella gracilis*
- *Amphorella cf cimensis*
- *Cylichnidia ovuliformis*
- *Boettgeria lowei*
- *Heterostoma paupercula*
- *Geomitra coronata*
- *Spirorbula obtecta*
- *Spirorbula depauperata*
- *Caseolus compactus* (large)*
- *Caseolus compactus* (small)*
- *Caseolus consors*
- *Caseolus abjectus agg.*
- *Caseolus subcalliferus subcalliferus.*
- *Caseolus hartungi*
- *Caseolus calculus*
- *Caseolus punctulatus*
- *Actinella effugiens*
- *Discula rotula*
- *Discula bicarinata*
- *Discula oxytropis*
- *Discula cheiranticola*
- *Discula calcigena*
- *Discula pulvinata*
- *Discula attrita*
- *Discula bulweri*
- *Discula tectiformis*
- *Pseudocampylaea portosanctana*
- *Leptaxis nivosa*
- *Leptaxis wollastoni*
- *Lampadia webbiana*
- *Helix subplicata*

Present, extant, non-endemic:
- *Theba pisana*

Absent, extant, endemic, not fossil:
- *Leiostyla corneocostata*
- *Leiostyla relevata*
- *Leiostyla ferraria*
- *Amphorella tuberculata*
- *Amphorella terebella*
- *Eucobresia media*
- *Lemniscia michaudi*
- *Discula echinulata*
- *Discula turricula*
- *Leptaxis erubescens*

Absent, extant, endemic, fossil:
- *Cecilioides eulima*
- *Discula leacockiana*
- *Discula testudinalis*

Absent, extant, non-endemic:
- *Vitrea contracta*
- *Oxychilus alliarius*
- *Cecilioides acicula*
- *Rumina decollata*
- *Balea perversa*
- *Cochlicella acuta*
- *Cochlicella barbara*
- *Caracollina lenticula*

*Some forms of *C. compactus*, for example *C. compactus areiensis*, appear to be extinct. Slugs, all non-endemic, are excluded.

Table 1. Species unique to samples at Site 1 and S3, at Fonte da Areia. Asterisked species were found only in late Holocene deposits not sampled at S3 (1-6 and 1-7).

Species Site 1 only	Nos.	Species Site S3 only	Nos.
Amphorella oryzae	5	*Staurodon saxicola*	2
Caseolus consors	3*	*Leiostyla subcorneocostata*	1
Actinella crassiuscula	21	*Leiostyla calathiscus*	8
Discula cheiranticola	1*	*Phenacolimax portosanctanus*	27
Pseudocampylaea portosanctana	20	*Caseolus calculus*	18
Pseudocampylaea lowei	4	*Discula bicarinata aucta*	3
		Discula calcigena	2
		Discula pulvinata	1
Total shells recovered	2121	Total shells recovered	1830

Among endemics, only three extinct species recorded for the island were absent from our samples. Of the 13 extant endemic species not found, only three have previously been found subfossil; most of the rest are restricted to rocky hills, or have otherwise limited distributions away from our sites. Only three non-endemic species were recorded in our samples. Two of them, *Punctum pygmaeum* and *Plagyrona placida* are recorded here for the first time on Porto Santo; they are unknown in the modern fauna. As on Madeira (Goodfriend et al., 1996), their presence predates human colonisation, and like the locally extinct endemics *Craspedopoma mucronatum* and *Staurodon saxicola,* they both survive on Madeira. *Theba pisana,* by contrast, is common and widespread. Its arrival is thought to follow human occupation of the island in the early 15th century AD.

Inspection of the appendices shows that the number of taxa recorded at each site is related to the number of samples made, which reflects both total number of specimens collected and the time spent collecting. Individual samples also vary in the number of taxa found, which is correlated with the total number of shells in each sample. Absences may therefore be caused by inadequate sampling, especially in individual samples. Even at site level, there are differences. For instance, the faunal composition of Sites 1 and S3, which represent the same stratigraphical sequence, are very close to each other, and have large numbers (Table 1). Two species found only at Site 1 were recorded only in Holocene deposits (1-6 and 1-7) not sampled at Site S3, where fossil *P. portosanctana* and *P. lowei* were loose on the surface. The larger number of species found at Site S3 show both that very rare species may be missed by accident, and that the smallest species may be missed when a sample is obtained by hand collecting from the substrate (Site 1) rather than sieving (Site S3).

Geographical Pattern

The modern distribution of snails on Porto Santo shows geographical differentiation (Cameron et al., 1996b). Site results have therefore been amalgamated on a regional basis (Fig. 1) to reflect this, and also to reduce the effect of sampling error (Table 2). Note that the inclusion of many sites with one or two samples in the SW region does not have much influence on the overall fauna recorded. The two offshore island samples included here (8 and 11) contain no species not found elsewhere. While the aggregate fossil faunas of all regions are similar, the Nei indices of similarity (Table 3) show SW and NC to be most similar, followed by the SE and SSE regions. 19 species are found in all four regions, and a further 16 are found in both the aggregated regions. Table 4 shows the remaining species with restricted, but coherent distributions. The SW region has many more unique species than the remainder, and the SW and NC regions combined have more than the SE and SSE combined. There is, however, a big difference in the character of the unique species; in the SW/NC area, 10 of the 15 are extinct, whereas only one out of eight is extinct in the SE/SSE area. In our samples seven species are unique to site S2 (SW region); six of these are extinct: the non-endemics *Punctum pygmaeum* and *Plagyrona placida,* the endemics *Leiostyla espigaoensis* and *Actinella morenensis* for which this is the type and only known locality (Seddon, 1990), and the endemics *Phenacolimax crassus* and *Geomitra acarinata*, known from earlier work.

Table 2. The occurrence of fossil taxa in each region of Porto Santo. Note that *Theba pisana* was present in all four regions, but is excluded from formal analyses, as it appears to be an introduced species.

Species/Sites	SW	NC	SE	SSE
Craspedopoma mucronatum	1	1		
Truncatellina linearis	1	1		1
Staurodon saxicola	1	1		1
Leiostyla subcorneocostata	1	1		
Leiostyla espigaoensis	1			
Leiostyla calathiscus		1		1
Leiostyla monticola		1	1	1
Punctum pygmaeum	1			
Plagyrona placida	1			
Phenacolimax crassus	1			
Phenacolimax portosanctanus	1	1	1	
Amphorella melampoides	1		1	1
Amphorella triticea	1	1	1	1
Amphorella oryza	1	1	1	1
Amphorella gracilis			1	
Amphorella cf. *cimensis*	1			
Cylichnidia ovuliformis	1		1	1
Boettgeria lowei	1		1	1
Heterostoma paupercula	1	1	1	1
Geomitra coronata	1	1	1	1
Geomitra acarinata	1			
Spirorbula obtecta				
Spirorbula depauperata	1	1	1	1
Caseolus compactus (large)	1	1		
Caseolus compactus (small)	1	1	1	1
Caseolus cf. *compactus vigiae*	1	1	1	1
Caseolus consors	1	1	1	1
Caseolus abjectus agg.	1	1	1	1
Caseolus subcalliferus submajor	1	1	1	
Caseolus subcalliferus subcalliferus	1	1	1	1
Caseolus hartungi	1			
Caseolus calculus		1		
Caseolus punctulatus	1	1	1	1
Caseolus bowdichianus	1	1	1	1
Actinella arcinella pap.	1	1	1	1
Actinella crassiuscula	1	1		
Actinella effugiens	1		1	1
Acinella morenensis	1			
Discula rotula			1	
Discula bicarinata			1	
Discula bicarinata aucta	1	1	1	
Discula oxytropis			1	1
Discula cheiranticola	1	1	1	
Discula calcigena	1	1		1
Discula pulvinata	1	1	1	
Discula attrita	1	1		
Discula bulwerii				1
Discula cockerelli				1
Discula tectiformis	1	1	1	1

(continues)

(continued)

Species/Sites	SW	NC	SE	SSE
Pseudocampylaea portosanctana	1	1	1	1
Pseudocampylaea lowei	1	1	1	1
Leptaxis sp.	1			
Leptaxis chrysomela		1	1	
Leptaxis cf. *fluctuosa*	1	1	1	1
Leptaxis nivosa	1	1	1	1
Leptaxis wollastoni			1	1
Lampadia webbiana			1	
Helix subplicata	1	1		1
Caseolus hartungi	1			
Caseolus calculus		1		
Caseolus punctulatus	1	1	1	1
Caseolus bowdichianus	1	1	1	1
Total No. of taxa	46	37	36	33
Total No. of samples	31	23	16	6

Changes with Time: the Fauna Overall

Using the A/I data, relating undated to dated samples stratigraphically, and using the presence of *Theba pisana* as an indicator of very recent age, we can allocate samples to age categories. Two dividing lines have been used: at A/I value of 0.2, separating all but the earliest Holocene from older deposits, and at A/I = 0.4, separating the last cold phase of the Pleistocene from earlier periods including warm interglacials with high sea levels. For some analyses, we use a further division at A/I = 0.7, to study the contribution of the oldest faunas at Sites 1, S3 and 7 to temporal and geographic patterns. Table 5 shows species by their temporal ranges within the deposits. Excluding the introduced *T. pisana,* there are 58 taxa in the array. 55 of these are found in the earliest division (A/I >0.4), assuming that material from S2 is assigned to it, 43 between A/I 0.2 and 0.4, and 31 from Holocene deposits (A/I < 0.2). There is thus a decline in observed richness with time. The occurrence of extant species in either or both of the two earlier divisions, but not in the Holocene, however, tells us that the examined fossil record is incomplete. Conversely, two species are restricted to Holocene deposits, and one is found only in the A/I range 0.2–0.4. All three are extant, small and rare as fossils. One other extant species, *Caseolus hartungi*, is present before A/I 0.4 and after A/I 0.2 but not between. It was found in small numbers in only two samples. 29 species, 50% of the total, occur in all three divisions, including the most abundant species in each. Eight of these are, however, extinct.

Assuming samples from S1 and S2 are at least A/I > 0.4, we can conclude that at A/I > 0.4, 22 are extinct (40%); at A/I 0.2–0.4, 11 are extinct (25.6%), and at A/I < 0.2, 8 are extinct (25.8%). Given the great disparity in times covered by each division it is apparent that extinction rate within the Holocene is far greater than in earlier periods. We note that if S1 and especially S2 represent younger deposits, the rates of extinction in later divisions would be even higher.

In the earliest deposits (A/I > 0.7, only present in the SW and NC regions: Sites 1, S3, and 7) 32 species are recorded, of which 11 (34.4%) are extinct. Fifteen species recorded at A/I > 0.4 (inclusive of these early samples, but excluding S2) are thus missing, but six of these are not known at all from the SW or NC regions, either fossil or modern. Samples within A/I 0.4–0.7 are found in all regions; if we remove the samples (A/I > 0.7),

Table 3. Values of the Nei index of faunal similarity in between region comparisons.

	NC	SE	SSE
SW	80.0	68.5	68.3
NC		71.2	70.5
SE			77.3

Table 4. Species unique to each region, and to the two geographically coherent pairs of regions. Asterisked species are extinct on Porto Santo.

SW	NC	SE	SSE	SW and NC	SE and SSE
*L. espigaoensis**	*C. calculus*	*A. gracilis*	*D. bulweri*	*C. mucronatum**	*D. oxytropis*
*P. pygmaeum**		*D. rotula*	*D. cockerelli**	*L. subcorneocostata**	*L. wollastoni*
*P. placida**		*D. bicarinata*		*C. compactus* (large)	
*P. crassus**		*L. webbiana*		*A. crassiuscula**	
A. cf. *cimensis*				*D. attrita*	
*G. acarinata**					
C. hartungi					
*A. morenensis**					
Leptaxis sp.*					

there are 47 species (still excluding S2), of which 16 are extinct (34.0%). At the other end of the scale, no Holocene deposits were sampled in the SSE region (Site 12). Only two species were unique to the region (Table 4), and only one of these is extinct (*D. cockerelli*).

Table 5. Temporal distribution of native species. Species extinct on Porto Santo are asterisked. Note that for extant species only the fossil ranges are shown. Species with a question mark against them are known from S2, but from no samples with A/I ratios <0.7. Otherwise, all sites except S1 and S2 are included.

Species	>0.7	>0.4	>0.2	<0.2	Species	>0.7	>0.4	>0.2	<0.2
*C. mucronatum**	x	x	x		*C. s. subcalliferus*		x	x	x
*T. linearis**	x	x	x		*C. hartungi*		x	x	x
*S. saxicola**		x			*C. calculus*		x	x	
*L. subcorneocostata**	x				*C. punctulatus*	x	x	x	x
*L. espigaoensis**	?				*C. bowdichianus**	x	x	x	x
L. monticola	x	x	x		*A. arcinella**	x	x	x	x
L. calathiscus			x		*A. crassiuscula**	x	x		
*P. pygmaeum**	?				*A. effugiens*	x	x	x	x
*P. placida**	?				*A. morenensis**	?			
*P. crassus**	?				*D. rotula*		x	x	x
*P portosanctanus**	?	x	x	x	*D. bicarinata*				x
A. melampoides	x	x			*D. b. aucta**	x	x	x	
A. triticea	x	x	x	x	*D. oxytropis*		x	x	
A. oryza	x	x	x		*D. cheiranticola*	x	x	x	x
A. gracilis				x	*D. calcigena*		x	x	
A. cf. *cimensis*	?				*D. pulvinata*		x	x	
C. ovuliformis	x	x	x		*D. attrita*	x	x	x	x
B. lowei	x	x	x		*D. bulweri*		x	x	
H. paupercula	x	x	x	x	*D. cockerelli**		x		
G. coronata	x	x	x	x	*D.tectiformis*	x	x	x	x
*G. acarinata**	?				*P. portosanctana*	x	x	x	x
S. obtecta	x	x	x	x	*P. lowei**	x	x	x	x
S. depauperata	x	x	x	x	*Leptaxis* sp.*	?	x		
C. compactus (large)	x	x	x	x	*L. chrysomela**		x	x	x
C. compactus (small)	x	x	x	x	*L.* cf. *fluctuosa**	x	x	x	x
C. cf. *compactus vigiae**	x	x	x	x	*L. nivosa*	x	x	x	x
C. consors		x	x	x	*L. wollastoni*		x	x	x
C. abjectus agg.	x	x	x	x	*L. webbiana*		x	x	
*C. subcalliferus submajor**	x	x	x	x	*H. subplicata*	x	x	x	x

The distinctive environmental division is at A/I 0.2–0.4, corresponding to the last cold period of the Pleistocene. The earlier section spans a much longer period including both warm and cold phases, while later samples come from the warmer part of the Holocene. In terms of presence and absence, there is no significant difference between the two pre-Holocene periods. Quantitative differences between the three temporal divisions in the five major GAG sites reveal no consistent patterns; troughs in relative abundance of particular species in some sites are countered by peaks in others. At Site 1 and Site S3, essentially from the same locality, we can compare changes between samples from A/I 0.4–0.7 and those from A/I 0.2–0.4. For the 17 species common to both sites in these age ranges, eight show concordant changes in relative frequency, and nine are discordant. In the best quantitative series from Site S3 there is much variation between samples in species proportions, but the only evident temporal trends are the relative reduction of large *C. compactus* agg. in the cold phase (A/I 0.2–0.4), accompanied by the first appearance of the extinct small *C. compactus areiensis.*

Changes with Time: Regional Patterns

As shown above, there are regional differences between faunas. Table 6 shows species richness data in the sites with more than two samples, ordered by the A/I divisions. Overall, the data show that, in early times, regional faunas were similar in richness though differing in composition, but later the fauna of the SW, and to a lesser extent that of the NC, declined, while that of the SE remained high, with some turnover in species composition. Seven out of nine extinct species survived into the Holocene at Site 2; four out of ten did so at Site 1, but only two out of ten did so at Site 7. At Site S3 (NC), there are no Holocene samples, but 10 out of 11 extinct species survived there into the last glacial (A/I 0.2–0.4).

The modern fauna of the island is now very well known (Cameron et al., 1996b; Seddon, in press). It is therefore possible to compare past and present distributions of extant taxa. The following patterns occur:

Continuously Broad Distribution: There are numerous species in this category, for example, *Heterostoma paupercula*, *Boettgeria lowei*, *Leptaxis nivosa*, *Spirorbula obtecta*, *S. depauperata, Caseolus punctulatus* and *Discula tectiformis*. Some of these are missing from the more grossly disturbed areas today.

Continuously Restricted Range: A number of species with restricted eastern distribution today seem to have been so restricted through

Table 6. Species richness and sample sizes, by A/I divisions, for the sites containing more than two samples. Unique indicates species confined to that division within the site.

Region/Site	>0.7	>0.4	>0.2	<0.2	Total	Region/Site	>0.7	>0.4	>0.2	<0.2	Total
SW Site 7						NC Site 1					
Samples	4	2	2	1	9	Samples	4	2	2	2	10
Shells	1310	266	454	526	2557	Shells	1329	248	156	388	2121
Species	30	15	14	13	32	Species	25	20	14	16	29
Extinct	10	5	2	2	10	Extinct	9	7	4	4	10
Unique	12	0	2	0		Unique	7	0	0	2	
SW Site 6						NC Site S3					
Samples		6	2		8	Samples	5	2	4		11
Shells		1261	354		1615	Shells	669	300	861		1830
Species		21	14		22	Species	21	20	26		31
Extinct		5	4		5	Extinct	6	6	10		11
Unique		8	1			Unique	1	1	6		
SSE Site 12						SE Site 2					
Samples		5	1		6	Samples		8	2	6	16
Shells		784	164		948	Shells		1494	254	775	2523
Species		28	24		33	Species		31	27	26	36
Extinct		5	3		7	Extinct		8	7	7	9
Unique		9	5			Unique		6	0	4	

the period covered by our samples. *Lampadia webbiana* occurs only at Site 2, near its modern location on Pico do Concelho, and *Discula oxytropis* only at Sites 2 and 12. *Leptaxis wollastoni* was recorded as abundant by Wollaston (1878) on Pico do Concelho, and it has been found there more recently by Hemmen & Groh (1986), but is unknown elsewhere, other than on the remote Ilhéu de Fora to the northeast. It is present fossil at Site 2 nearby. *Discula rotula* and *D. bicarinata* have somewhat more extensive distributions in the east of the island only and are fossil at Site 2. *D. bulweri*, recorded at Site 12, is also limited to the east. *D. calcigena* occurs everywhere except the NE both as fossils and in modern faunas. At the western end of the island *Leiostyla subcorneocostata* is present in early horizons at Site 7, in Site S2, and in one of Seddon's samples from Fonte da Areia (Site S3). It appears always to have been rare. The very similar *L. corneocostata* lives today at the southwestern tip of the island near Site 7.

Reduction of Range: Apart from outright extinction, the most dramatic reductions in range and abundance are shown by *Geomitra coronata,* abundant in the fossil record, but now confined to two widely separated localities, and *Helix subplicata,* not as abundant, but widespread, which is now confined to the offshore islet Ilhéu do Baixo in the southwest. *Pseudocampylaea lowei,* once widespread, but now extinct, survived into the 20th century on Ilheu de Cima in the SE, while *P. portosanctana,* once widespread, now has a disjunct distribution, mainly on offshore islets (Cameron and Cook, 1996). *Discula attrita*, now mainly on Pico da Ana Ferreira, is present throughout Sites 7, 6 and 1. *Amphorella triticea* was recorded at all fossil sites but is now an eastern species only. *Caseolus consors* and *Discula cheiranticola* also occur as fossils in the SW and NE, but are now confined to the east.

Expansion of Range: Modern endemic species not found in the fossil record were considered earlier. It seems unlikely that their ranges have increased significantly. *Caseolus hartungi*, however, is widespread (mainly coastal) today but occurs very rarely, and in small numbers, in fossil samples from the SW only.

Change of Location: *D. pulvinata* is now established near Site 1 and is present only in the youngest Site S3 samples from this area. It is not now known from near Sites 2 and 7 where it occurs as a fossil.

Morphological Variation

Fossil snails are, necessarily, identified by shell characters, and those that are extinct can only be described from such characters. Some species have "forms" or subspecies known only as fossils. The fact that there may be variation in time adds to the problems of determination Amongst the many morphometric studies that could be made using our material, we have undertaken three, illustrating different aspects of variation.

(1) Variation in *Heterostoma*. A small discoidal helicid with a down-turned mouth, unlike anything else on the islands, occurs on all three Madeiran groups. It is referred to here as *Heterostoma paupercula* (Lowe, 1831). Since Lowe's time it has been divided into three species in two genera on the basis of size, shell sculpture and colour, presence or absence of a tooth in the aperture and presence or absence of an epiphallus. When these distinctions were made (Mandahl-Barth, 1943), *H. paupercula* was redefined as hemiphallic, with a toothed aperture and small, granular and brown shell. A new genus *Steenbergia duplex* was euphallic, with no tooth and a paler shell with larger granules. Two subspecies were named, which differed in size and location, later regarded as separate species by Waldén (1983). Even at that time it was apparent that the characters do not associate consistently (Backhuys, 1975; Waldén, 1983; Lace, 1992; Cameron et al., 1996a), so that for practical convenience we have used the original name. Lace (1992) studied animals from the three island groups within the archipelago. She found considerable variation in size and proportions of genitalia throughout the range. On Ilhéu Cima, at the eastern end of Porto Santo and the neighbouring mainland in the region of the present Site 2 the populations contained small, euphallic toothed individuals and larger hemiphallic individuals lacking the tooth. Studies of isozymes showed that there was reduced heterozygosity and similar but different frequencies of alleles in the two types. Cook & Lace (1993) suggested that the hemiphallic individuals might be part selfing, part female, while the euphallics are cross-fertilizing. Craze & Lace (2000, 2002) found populations composed entirely of hemiphallics, which is not

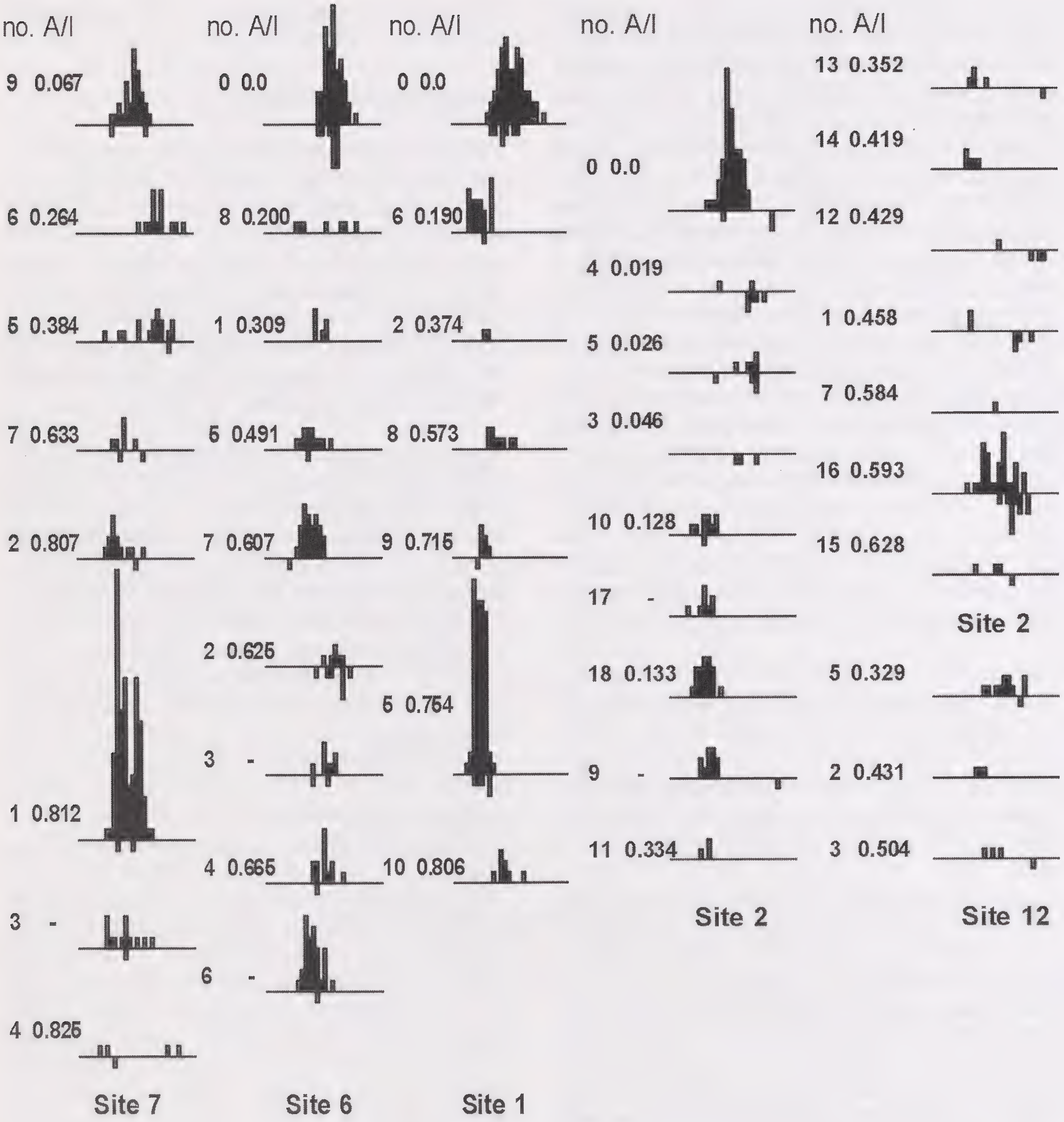

FIG. 4. Shell breadth in *Heterostoma paupercula* from the five sampled sites. Left: from SW and NC, right: samples from SW. Shells measured to 0.1 mm; range shown is 3–6 mm. Toothed individuals above horizontal line, untoothed individuals below line. Samples given an A/I ratio of 0.0 are modern.

possible if they simply act as females, and molecular differences between hemiphallics and euphallics. The different types may therefore be separate sympatric species, although the possibility that the genetic differences are due to partial selfing has yet to be excluded. Elsewhere on Porto Santo untoothed shells are euphallic and the same size as toothed (Lace, 1992; Cameron et al., 1996a). Frequency of tooth presence, shell size and colour vary in a seemingly irregular manner from sample to sample. The relations now seen in the eastern region appear to be limited to it.

In order to examine whether these patterns of distribution also occur in the fossil sequences, and whether any changes have taken place, all shells from the samples have been measured and scored for presence or absence of tooth. Shell colour proved impossible to score. The results are summarized as a series of histograms (Fig. 4). At Sites 7, 6 and 1 untoothed individuals are present but quite rare (6.6% over all fossil samples) and are as likely to be smaller as larger than toothed individuals. Untoothed individuals are more common (23.4%) in the long series from Site 2. They are usually larger than toothed individuals, especially in the early part of the sequence. The mean breadth in the toothed samples appears to drop from 2-16 (A/I = 0.593) to 2-10 (A/I = 0.128), and after the gap at 2-3 to increase. The majority of individuals in 2-1 and 2-12 (A/I 0.429, 0.458) are untoothed and large. Since mean breadth varies from sample to sample significance tests have been carried out within samples and combined. Taking only samples where there are at least two untoothed individuals the trend obtained by combining samples from Sites 7, 6 and 1 gives a normal deviate of 0.62, which is not significant. For Site 2 the normal deviate is 7.20 ($P << 0.001$), supporting the difference apparent in the diagrams. The present pattern, in which samples with large untoothed and small toothed shells are restricted to the SE, was therefore present throughout the recorded history of *Heterostoma*.

(2) *Spirorbula depauperata* and *S. latinea.* Wollaston (1878) listed three *Spirorbula* species for Porto Santo. One, *S. obtecta*, is clearly distinct, but the extinct *S. latinea* and the extant *S. depauperata*, which is also found as a fossil, are very similar. Examination of the type

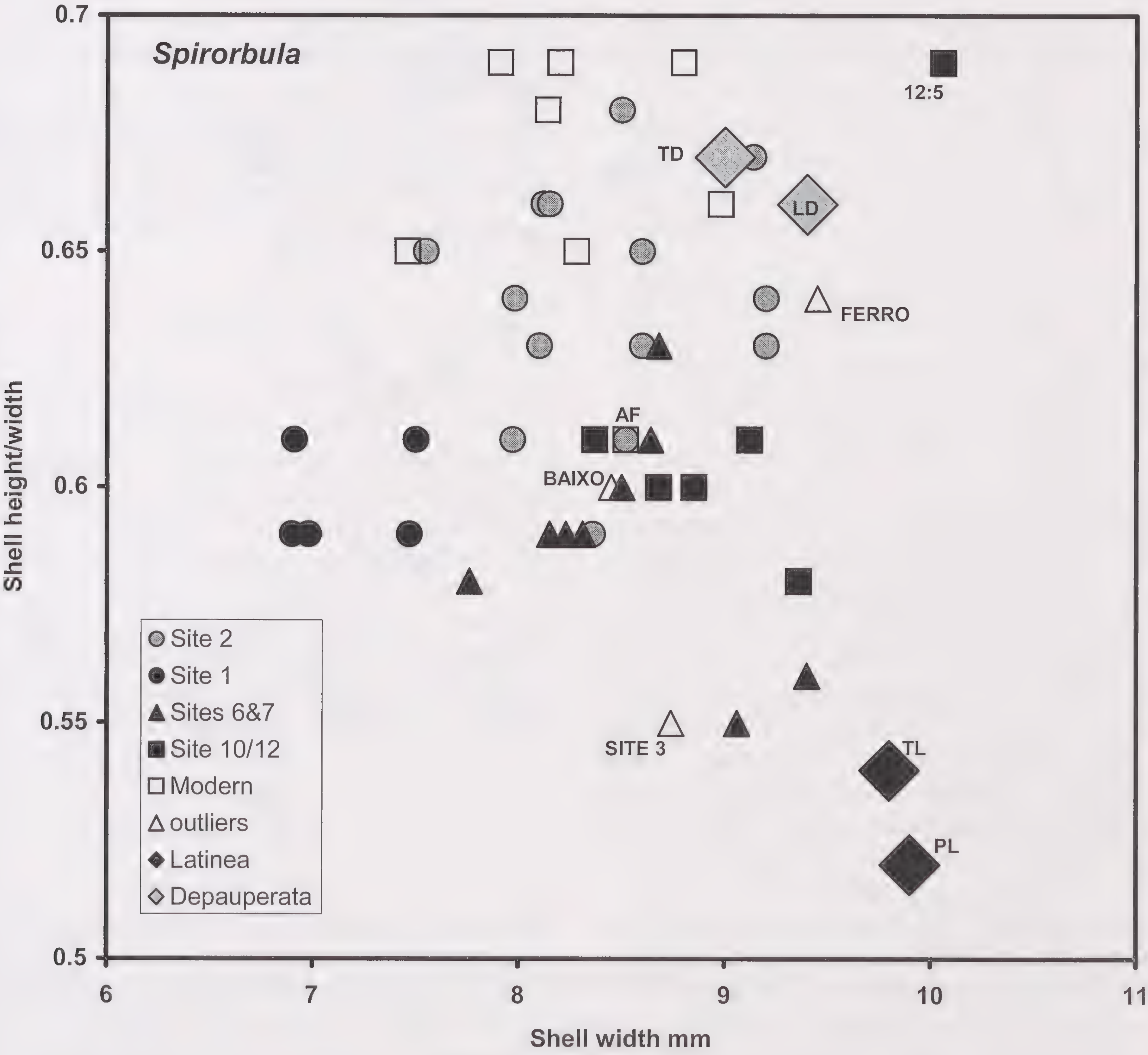

FIG. 5. The relationship of mean shell width and shell height/width ratio for samples of *Spirorbula depauperata/latinea.* For locality symbols, see inset. Diamonds indicate the positions of type specimens (TD = type of *S. depauperata*; TL = type of *S. latinea*), and of other samples made by the species' authors (LD = Lowe, *S. depauperata*; PL = Paiva, *S. latinea*). AF indicates the position of a modern sample from Pico da Ana Ferreira. Fossil samples from minor sites labelled.

specimens alone suggests that the distinction is relatively easy to make. However, examination of our material and of modern samples of *S. depauperata* shows that this is not the case. Fig. 5 shows the relationship between shell size and shape for the types, for museum lots collected in the nineteenth century, and for recent and fossil samples made by us. The whole range of shapes is found. There is evidence of geographical variation in size and shape among fossil samples, but no consistent trends with time within each site (data not shown). The types and early collections lack precise locality data. All but one of the modern samples come from the east, and they overlap with fossil samples from Site 2. The outlying position of the sample 12-5 is discussed further below.

(3) Variation in *Discula oxytropis.* The modern form of this species is restricted to the south-eastern part of Porto Santo. Wollaston (1878) described a variety (α *subcarinulata*), common as a fossil, characterised by larger size and relatively higher spire. He also noted the fossil occurrence of *D. vermetiformis* (Lowe, 1854) as a closely related species characterised by a double keel, a character that it shares with *D. bicarinata* and its close relatives. Waldén (1983) treated both these as extinct subspecies of *D. oxytropis.* Fig. 6 shows the relationship between shell size and shape, and the extent of double keeling, for the types and other old museum collections, and for fossil and modern samples made by us. Early samples are not localised; our own fossil material comes only from the east, in or

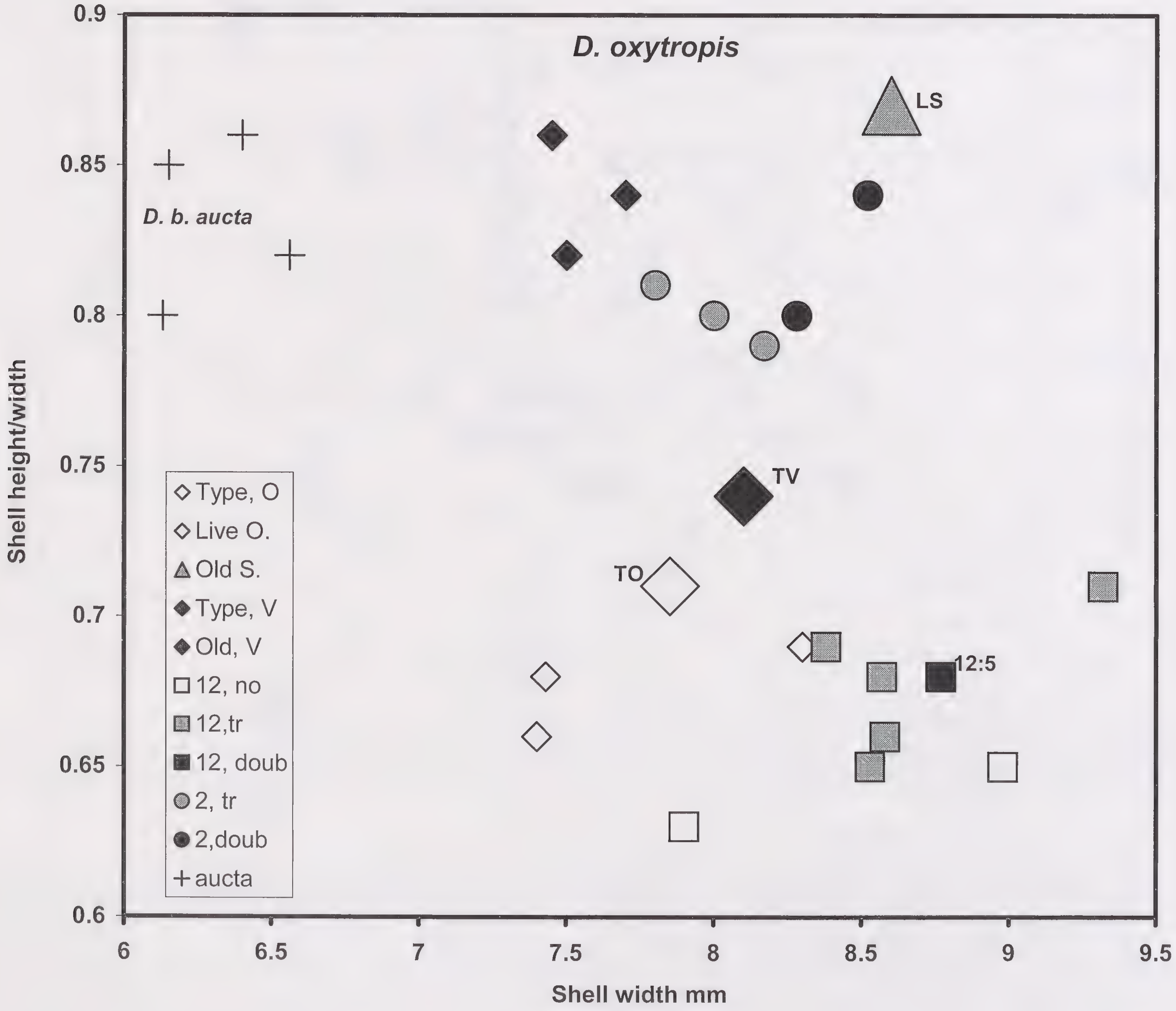

FIG. 6.The relationship between mean shell width and shell height/width ratio for samples of the *Discula oxytropis* group. For symbols, see inset (T = type specimen; L = sample labelled *subcarinulata* by Lowe; O = *D. oxytropis*; V = *D.* cf. *vermetiformis*; S = *D.* cf. *subcarinulata*). Open symbols, single keel; grey symbols, trace of second keel; black symbols, double keel. The crosses show the position of fossil samples of *Discula bicarinata aucta* (see text).

near the present distribution. The figure also shows the same data for fossil samples of *D. bicarinata aucta*, the form Wollaston regarded as the closest to *D. vermetiformis.*

As with *Spirorbula* above, consideration of many samples blurs the apparently clear separation suggested by the types. At Site 12, the whole range from clearly single to clearly double keeled specimens can be found, and, again, sample 12-5 is distinctive. Given that Site 12 samples were scattered, and not in a single stratigraphical column, we suspect that 12-5 represents a distinct fauna. The modern distribution of *D. oxytropis* is in much the same area. Samples from Site 2, nearby, generally resemble the old sample of *D. o. subcarinulata* to a greater extent, with mixed expression of keeling, while the nineteenth century collections labelled *D. vermetiformis* resemble *D. o. subcarinulata* in shape more than does the type. *D. bicarinata aucta* seems clearly distinct on the basis of size. Shape appears to vary geographically, while keeling is idiosyncratic. In neither case is there any consistent relationship with age, except that all modern shells are single-keeled and relatively low spired.

DISCUSSION

The reasons for high diversity and rapid evolution on islands are still incompletely understood. Our survey of extant faunal distributions in the Madeiran archipelago has been directed at these problems. Knowledge of the fossil associations adds a historical dimension. Results presented here suggest that faunas on Porto Santo and Madeira have responded differently to past climatic change, that there have been different levels of extinction and that different island topography provides a central explanatory factor.

Stasis and Change

The predominant impression is one of stasis, even at regional level within the island. Not only do the most abundant species remain dominant throughout the sequences, but it is also hard to relate extinctions and fluctuations in abundance to known climatic variation in the Quaternary. Only comparison of the fossil fauna as a whole with the modern one shows profound change. Even then, geographical distributions remain unchanged in many species, and in others reduction in modern range can be attributed to human disturbance. The morphometric studies support the pattern of geographical and temporal stasis; local differences in character associations in *H. paupercula* are maintained; a geographical pattern of shell shape is maintained in *S. depauperata*, and only in *D. oxytropis* do some characters appear to fluctuate within localities.

In these respects the fossil faunas of Porto Santo differ from those of the only fossiliferous site on Madeira, situated on its eastern peninsula, the Ponta de São Lourenço (Cook et al., 1993; Goodfriend et al., 1994, 1996). On Madeira, there were substantial shifts in relative abundance and occurrence of species with time. In apparently wetter phases, fossil faunas included larger numbers of species that have forest or montane distributions today; in drier phases species characteristic of dry, open and mostly coastal habitats prevailed. The difference between the modern and fossil faunas at the Madeiran fossil site is also far greater: only 12 out of 43 fossil species recorded now live on the peninsula. Of these, two, *Theba pisana* and *Cochlicella barbara*, are introduced species present only in samples from post-colonisation horizons.

The Madeiran fossil sequence spans a shorter period, most samples being from the Holocene, and most earlier ones from either the last interglacial or a warmer interstadial within the last cold phase (approximately 45 ka BP; Cook et al., 1993). Cold-phase conditions correspond to periods of rapid sand accumulation and an absence of snail fossils, a situation not evident on Porto Santo. Within the Holocene, but prior to human colonisation, there is a small shift from open to woodland species, terminated abruptly and reversed in post-colonisation samples. The very oldest samples (135+ ka) cannot be assigned to particular Pleistocene conditions; they have open habitat species comparable to those in post-colonisation samples. There are no natural forests surviving on Porto Santo and little indication as to what pre-colonisation vegetation was like, though it is clear that there was at least some tree and shrub cover. Three species fossil and extinct on Porto Santo are found today in forest faunas on Madeira: the endemic *C. mucronatum*, and the non-endemic but native *P. pygmaeum* and *P. placida.* They occur together in Site S2, suggesting a forested habitat, although in company with *S. saxicola*, also extant on Madeira, which is primarily an open habitat species.

Altogether, the evidence from this analysis suggests that the Porto Santan fauna remained, until human colonisation, much more stable through the late Pleistocene and Holocene than the equivalent fauna of the eastern peninsula of Madeira, which today resembles Porto Santo in substrates and habitats. There are no clear signals of response to climatic or habitat change.

Extinctions and Appearances

Cameron & Cook (1996, 1999) reviewed rates and causes of extinction on Madeira, Porto Santo and the Deserta Islands. The overall level of extinction of species known as fossils on Porto Santo was 15 out of 54 (27.8%). The present results, including endemics and the two native non-endemics, bring the totals to 25 out of 64 (39%), the increase, influenced slightly by taxonomic decisions, reflecting inclusion of recently described extinct species. The level of extinction is similar on Madeira, at 33% (Cameron & Cook, 1996). Two-thirds of this loss is human-induced and thus very recent (Goodfriend et al., 1994). Our dated samples from Porto Santo are mostly precolonisation, but because approximately 26% of species known to be present in the Holocene are now extinct, this too suggests a human cause.

In the case of Porto Santo we have a spread of geographical as well as temporal variation in composition. Impoverishment and extinctions appear to start well before the Holocene, largely due to a marked early decline in the SW, where a number of species unique to that region disappeared quite early in the sequence. Whereas in fossil samples, especially in early ones, the SW is the richest region, in the modern fauna the situation is reversed: the east has more restricted species and richer faunas than to the west (Cameron et al., 1996b). Today, the greater heights and extent of the eastern hills, and the larger areas free of mobile sand, make this difference understandable in terms of refugia and range of habitats. While we lack very early samples from the SE and SSE regions, those from the NC region, including some of the earliest in our array, maintain high levels of richness until the Holocene, but lack a significant suite of unique species as once found in the SW.

On Porto Santo, very few species are missing from the earliest major temporal division (A/I > 0.4). All are small and are rare in the samples and their absence may well be due to sampling error. On Madeira, however, a significant number of species appear for the first time relatively late in the sequence, and in sufficient numbers to suggest that we are not dealing with sampling artefacts. Thus, for example, *H. paupercula* and *C. bowdichianus,* found throughout the Porto Santan series, do not occur until the Holocene on Madeira, and several other, later abundant, species such as *Geomitra delphinula, Spirorbula squalida, Actinella promontoriensis* and *Discula polymorpha* are missing from the earliest samples (before approximately 135 ka), and are very rare before the Holocene. The last, a very close relative of *D. calcigena* on Porto Santo, is particularly interesting, as it is typical of open, dry habitats of the kind indicated as being represented by the earliest Madeiran samples. Because there is a long temporal gap, from approximately 8 ka to approximately 45 ka, in the Madeiran sequence, the timing of these arrivals remains uncertain, but Porto Santo is a likely source, especially for *C. bowdichianus* and *H. paupercula.* There are very few extant native species in common between the two islands, and a different balance of genera and species richness within each (Seddon, in press). The similarity is slightly greater when extinct species are considered, a point first made by Charles Lyell in a letter to Wollaston in 1856 (Wilson, 1970). The depth of sea between the two islands would seem to exclude a land connection at any time, but there has been a continuing, if very small exchange, some effects of which have been removed by human-induced extinctions.

Patterns and Topography

Porto Santo is an older island than Madeira. Volcanic activity ceased at least 8 million years ago, the topography became strongly eroded and extensive wind-blown deposits have accumulated. This contrasts with Madeira, where there have been repeated phases of vulcanism, possibly down to a quarter of a million years ago, with lava sometimes occupying existing river valleys (Nascimento Prada & Serralheiro, 2000). Unlike Madeira, where coasts shelve steeply into deep water, Porto Santo is surrounded by a substantial platform of shallow sea. At Pleistocene low sea levels most of this was exposed to make the island at least three times its present size and include the offshore islets (Seddon, 1995). Considering the patterns of distribution in the modern fauna, Cameron et al. (1996b) suggested that small-scale allopatric divergence had resulted from periodic bursts of sand mobility, which isolated populations on the rocky hills. Changes in sea level that were probably the trigger for sand mobility, would isolate and reconnect offshore islets. Human disturbance has mimicked these effects, producing some vicariant distributions (Cameron & Cook, 1996). The results reported here show the time scale to be long, since the patterns in the modern fauna are mostly repeated in the fossil faunas. This reflects a growing consensus that, away from the immediate proximity of glaciers, differentiation of modern faunas started quite early in the Pleistocene (Weir & Schluter, 2004).

On Madeira there is a sharp contrast between relatively undifferentiated forest faunas and those from drier coastal scrub, which have a number of geographically distinct species associations, including replacing sister species (Cameron & Cook, 2001). The differences between forest and coastal scrub are deep, reflecting differential colonization or perhaps an early evolutionary radiation. As on Porto Santo, geographical differentiation in the open, coastal environments can be explained by periods of isolation. These could result from volcanic activity, extension of the forest zone to sea level during colder or wetter phases and/or sea level change during the Pleistocene. The isolating processes appear to have modified the structure of the islands at rates that happen to match rates of dispersal of taxa in such a way as to facilitate evolutionary divergence.

The large changes in faunal composition in the Madeiran fossil deposit, compared with those on Porto Santo, are noteworthy because the fossil site has no high mountains nearby, and is by far the driest part of the island, yet contains species with modern high montane distributions. Some open country species may be late arrivals from Porto Santo. That they did not arrive earlier might be chance; it might also indicate that earlier environments were unsuitable. Present climate and habitat is very similar to that of Porto Santo, so that, given the present topography, it is hard to see why it should have differed radically in the past.

We are left, therefore, to discover some other event or circumstance unique to one or the other island. One possibility is that the extreme east of Madeira has altered radically within the period covered by the fossil record. The north side of the Ponta de São Lourenço is a set of sheer cliffs descending into a deep-sea trough between Madeira and Porto Santo. The peninsula forms part of a ridge connecting Madeira to the Deserta Islands, the intervening part of which is now submerged, and it seems likely that submergence is a product of erosion or collapse as much as of changing sea levels. This ridge and the Desertas as a group are products of relatively recent volcanic activity. They rise from great depths on either side, and are remnants of a larger landform. At least one massive landslip has occurred on Deserta Grande in recent times, causing a minor tsunami in Funchal, the capital of Madeira. The Desertan snail fauna has many species in common with that of Madeira (Cameron & Cook, 1999). A greater and higher land mass rising on the north side of the peninsula but now lost by slumping could explain the past occurrence of montane and forest species on the peninsula. By comparison, no such events would have taken place on Porto Santo in the same time frame because of its position on a shallow submarine plateau.

Equally puzzling is the early impoverishment of faunas in the SW of Porto Santo. This could be due in part to the small number of samples and sites investigated in a widespread and complex series of deposits. We cannot help noticing, however, that the area concerned lies directly in the path of any tsunami emanating from a large scale collapse at the eastern end of Madeira, neatly complementing the suggested change there.

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APPENDIX 1

Details of the sites used in this study. The sequence retains GAG numbering, and numbers assigned to MBS sites, and run clockwise from the extreme SW (Ilhéu de Baixo). Site locations are shown in Fig. 1. Grid references are provided using the grid on the 1970 1/50,000 map published by the Portuguese Instituto Geográphico e Cadastral. Asterisked GAG sites are those with more than two samples, which are subject to detailed analyses.

Southwest Region (SW)

Site 8. Two localities on Ilhéu de Baixo (approximately 709525).

8-1. Clayey colluvium, slightly indurated. Sample taken of material between 32 and 45 cm from top of a gully exposure 65 cm deep.

8-2. Friable clay deposit, eroded to seaward. Sample from material 10 to 35 cm below top of unit, which is capped by a red buried soil.

Site 3. One locality at Ponta da Calheta (718549).

3-1. Sample 40 to 80 cm below the top of a 3.5 m deep deposit of amorphous carbonate-rich sand resting on volcanic bedrock. Above this deposit is a car park and recently disturbed material.

Site S2 (approximately 715556) about 1 km NE of Porto da Morena.

Samples S2-2 and S2-3 come from a 3.5 m deep exposure of colluvium on the NW side of a small valley. There are two units: a lower (2.2–3.5 m) one of yellow sand, in which S2-3 was made in the top 50 cm, and an upper (0–2.2 m), in which S2-2 was made in the central 1 m portion. S2-1 was made from an extension of this upper unit, approximately 300 m away

Site 7* (700563). Eroded slopes facing south to Ponta da Canaveira.

Samples 7-1 to 7-5 come from a single, trackside exposure approximately 18–20 m deep. There are two units. The lower one, resting on bedrock, varies between 3.5 and 5 m deep, is colluvial, and varies in texture from brown-red clays to patches with many rocky inclusions. 7-1 and 7-2 come from 50 cm vertical sections near the base of this unit, and 7-3 from a 25 cm section starting 75 cm from its top. The upper unit, approximately 15 m thick, is sandier, with later fissure fills. 7-4 is a 40 cm section starting 80 cm from the base of this upper unit, and 7-5 a similar section starting 3.1 m above the base of this unit. A/I ratios indicate that 7-1, 7-2 and 7-4 are all much the same age, and very old, whereas 7-5 is much younger. The remaining samples (7-6 to 7-9) come from separate exposures slightly higher up the valley, and we cannot integrate their stratigraphical details. On A/I ratios, 7-7 and 7-8 are older than 7-5, but younger than 7-1 to 7-4. 7-6 and 7-9 are much younger.

Site S1 (700563). Ponta da Canaveira.

Samples taken from a 19 m deep exposure of calcarenite with interbedded layers of sand or silt. S1-4 comes from near the base of this sequence (18.0–18.1 m), in a breccia containing shells and volcanic clasts. S1-3 (16.0–16.5 m) comes from a bed of consolidated yellow sand. S1-2 comes from a narrow band of silt at 5.45–5.50 m. S1-1 was made at the same depth, but from a short band of blown sand.

Site 11. Ilhéu de Ferro (689560).

Single sample collected by excavation from a approximately 30 cm thick band of indurated clay exposed by erosion. This sample is undated.

Site 6*. West-facing slopes of Espigão (705564).

Samples 6-1 to 6-5 come from the slope of an eroded gully, exposing approximately 7 m depth of sediments. Three units were distinguished. 6-5 represents picked shells from a band 10 cm deep, just below the top of the basal, red sandy clay unit, of which only approximately 30 cm were exposed. 6-4, 6-3 and 6-2, each approximately 50 cm deep, come, respectively from very near the base, two thirds of the way up, and just below the top of the middle unit, approximately 6 m depth of largely unbedded sands, slightly indurated, with evidence of later fissure fill near the top. 6-1, 30 cm deep, represents the whole thickness of the uppermost unit, which consisted of clayey sand, darker in colour than the sands of the unit below. A/I ratios for 6-4 and 6-2 are very similar, suggesting that the middle unit accumulated very rapidly. Some A/I values for 6-5 are anomalous, but the largest are similar to those of 6-4 and 6-2. 6-6 and 6-7 come from a much smaller exposure within 50 m of the previous one. 6-7 represents picking from a section 10 cm deep, 10 cm from the top of the lowest unit exposed (80 cm), which is traceable to the unit containing 6-5 (A/I determinations confirm this). 6-6 comes from a 30 cm deep unit of red clay immediately above the unit containing 6-7. No A/I determination is available for this sample. It appears to be stratigraphically equivalent to the unit containing 6-4 to 6-2, but the unit differs radically in thickness and composition. 6-8 comes from a road cut exposure, again close to samples 6-1 to 6-5. It represents the upper portion of a 50 cm deep unit traceable to the unit containing 6-1, and with the same character.

Site 9. Road cutting on slopes of Espigão, approximately 500 m inland from Site 6 (710561).

A single sample, picked out between 45 and 75 cm below the top of a 0.8 m deep exposure of loose, sandy colluvium resting on volcanic bedrock. There are no A/I data for this sample, but the presence of *Theba pisana* suggests that it is late, probably post 1420 AD.

Site 4. Eroded deposits above sea-cliffs at São Sebastião (718580).

Sample 4-1 comes from the bottom 10 cm of a 3 m unit of bedded and partly indurated sand, which overlies a layer of brown blocky clay containing concretions. A/I data indicate a similar age to the oldest samples from Site 6. 4-2, for which there is no A/I data, comes from a nearby exposure, but from the top 10 cm of a much younger deposit, probably similar to that containing samples 6-1 and 6-8.

North-Central (NC)

Site 5. An exposure at the bottom of a gully on the north side of Barbara Gomez (727602).

Goodfriend identified here a sequence of six well-stratified units exposed over approximately 6 m depth, and clearly earmarked the site for further work. 5-1 comes from the top of the uppermost unit; 5-2 spans 30 cm depth, 90 cm below the top of the fifth unit down. Many intervening units are marked as holding shells. A/I data for 5-2 equates it with 4-1, and with the earliest samples from Site 6. There are no A/I data for 5-1, but the stratigraphy suggests that it is much younger. It also contains *Theba pisana.* It should be noted that this site contains only species common to both SW and NC regions.

Site 1* (approximately 729613). Exposures in erosion surfaces and road cuttings at Fonte da Areia.

The main sequence of samples here can be related to a section varying in depth, but generally 30 m or more. This section is multilayered, with a number of narrow calcrete bands within it. The basal 10 m appeared not to contain shells. All samples except 1-6 and 1-7 came from this section. Figure 3 compares the stratigraphical positions of samples in this series with the range of A/I values. Samples 1-1, 1-2, 1-8, 1-3 and 1-10 show a coherent pattern, with only typical variation (Fig. 2) between individual A/I values. 1-5, 1-4 and 1-9 have wide ranges of A/I values (some of them extreme, A/I > 1.0), and the mean A/I values run counter to their stratigraphical position. The lowest sample, 1-5, comes from a dense accumulation of shells, clearly washed together. There appears to have been significant mixing and reworking of deposits in this sequence. Samples 1-6 and 1-7 come from exposures above, and 100–200 m away from the main section. They have similar, low A/I values, and, on topographical grounds, are probably aeolian deposits.

Site S 3. Fonte da Areia.

This section was made very close to the main sequence of Goodfriend. No A/I data are available for these samples, but it is possible to match up the stratigraphical diagrams with some confidence. They span the same depth, and have some well defined calcrete layers at approximately the same intervals. These are used to align the sequences as shown in Fig. 3. Other discontinuities, also shown, are impossible to align with certainty.

Southeast Region (SE)

Site 2* (centred on 792594).

Samples from this site come mainly from coastal and road cut exposures through sandy deposits at Calhau da Serra de Fora/Porto dos Frades. Many samples are single removals from particular exposures, and cannot be directly correlated with strata elsewhere; A/I values are the only means of ordering them. In particular cases where several samples come from a single stratigraphical column, the agreement is good (2-3 to 2-5; 2-9 to 2-12; samples 2-15, 2-16 and 2-7 were identified as coming from the same stratigraphical unit).

South-Southeast Region (SSE)

Site 12* (including Goodfriend's Sites 10 and13) (788587).

Samples come from clay and rocky colluvium along the coast between the new harbour (Peneda) and Pedras Altas immediately below Pico do Baixo. The deposits were impossible to align along this coast, due to strong dipping and inconsistent colouring, the material coming from steep hill slopes above, of varying character. 12-2 was 1.5 m above 12-1 in the same section, but has a very slightly larger mean A/I ratio. 12-1 to 12-4, and 10 all came from near the base of exposed sediments. 12-5 was collected higher up relative to sea level; above it was an unsampled unit containing *Theba pisana.* The limpet shells (Site 13) from the basal calcrenite just above sea level give a mean A/I ratio only marginally larger than those from some of the overlying colluvial deposits. It would appear that these deposits accumulated rather rapidly; the range of A/I values here is small.

APPENDIX 2

Taxa recorded in Site 7, samples arranged by A/I ratio and stratigraphical position. Note that, at this site, there is some discordance between the two (see Appendix 1).

A/I	0.825	0.812	n/a	0.807	0.69	0.63	0.384	0.264	0.067
Species/Samples	4	1	3	2	8	7	5	6	9
Craspedopoma mucronatum									
Truncatellina linearis		3				1			
Staurodon saxicola									
Leiostyla subcorneocostata		23	1	1					
Leiostyla calathiscus									
Leiostyla monticola									
Phenacolimax portosanctanus									

(continues)

(continued)

A/I	0.825	0.812	n/a	0.807	0.69	0.63	0.384	0.264	0.067
Species/Samples	4	1	3	2	8	7	5	6	9
Amphorella melampoides		11		4					
Amphorella triticea			2						
Amphorella oryza		6		5					
Amphorella gracilis									
Cylichnidia ovuliformis		12							
Boettgeria lowei		11	1	7					
Heterostoma paupercula	5	124	15	16	1	9	20	17	37
Geomitra coronata		67	50	19	1				30
Spirorbula obtecta		11	6	1			2	2	
Spirorbula depauperata	5	34	20	11	21	21	2		1
Caseolus compactus (large)	4	7	20	2	1		8		
Caseolus compactus (small)	23	114	86	21	18	40	29	13	72
Caseolus compactus vigiae	8				7	1			9
Caseolus consors								5	
Caseolus abjectus agg.	2	157	25	33	6	10	2		53
Caseolus subcalliferus submajor	10	12	14	8	17	27	21	19	15
Caseolus s. subcalliferus									
Caseolus hartungi									
Caseolus punctulatus	21	54	4	6	31	16	28	19	106
Caseolus bowdichianus	5		5	1	3	4			
Actinella arcinella pap.	1								
Actinella crassiuscula		14	5						
Actinella effugiens		36	4	7		1	1	2	61
Discula rotula									
Discula bicarinata									
Discula bicarinata aucta		21		4					
Discula oxytropis									
Discula cheiranticola	1	1							
Discula calcigena									
Discula pulvinata							77	46	
Discula attrita	1	5	1	2					7
Discula bulwerii									
Discula cockerelli									
Discula tectiformis	1	5		3	10		27	21	27
Pseudocampylaea portosanctana		2		1					26
Pseudocampylaea lowei		10		2					
Leptaxis sp.									
Leptaxis chrysomela									
Leptaxis cf. *fluctuosa*	1				5		3	4	
Leptaxis nivosa	9	26	1	11	13	2	47	39	82
Leptaxis wollastoni									
Lampadia webbiana									
Helix subplicata		10	1	11					
Theba pisana									
No. of taxa	15	25	18	22	13	11	13	11	13
No. of shells	97	776	261	176	134	132	267	187	526

APPENDIX 3

Taxa recorded at Site 6, samples arranged by A/I ratio and stratigraphical position. GAG data on numbers of *Caseolus bowdichianus* are missing. In sample 5 (asterisked) A/I values were exceptionally variable (Appendix 1).

A/I	0.806	0.754	0.754*	0.715*	0.663*	0.573	0.349	0.347
Species/Samples	10	3	5	9	4	8	1	2
Craspedopoma mucronatum								
Truncatellina linearis								
Staurodon saxicola								
Leiostyla subcorneocostata								
Leiostyla calathiscus								
Leiostyla monticola								
Phenacolimax portosanctanus								
Amphorella melampoides								
Amphorella triticea	1							
Amphorella oryza			37		4			
Amphorella gracilis								
Cylichnidia ovuliformis								
Boettgeria lowei								
Heterostoma paupercula	15	11	33	15	32	15	7	8
Geomitra coronata	1		39		16			
Spirorbula obtecta	14	3	2		4	14	8	14
Spirorbula depauperata	25		11		9			
Caseolus compactus (large)		5	70	22	31		5	3
Caseolus compactus (small)	89	52	54	20	52	56	58	47
Caseolus compactus vigiae								
Caseolus consors								
Caseolus abjectus agg.	10	16	19	2	2	2		
Caseolus subcalliferus submajor	60	30	2	30		3	20	19
Caseolus subcalliferus subcalliferus			3		4			
Caseolus hartungi								
Caseolus punctulatus	20	45	3	30	3	4	23	20
Caseolus bowdichianus	+			+	+	+	+	
Actinella arcinella	24	7	3	1		5	7	4
Actinella crassiuscula						2		
Actinella effugiens	8	1	1	1				2
Discula rotula								
Discula bicarinata								
Discula bicarinata aucta								
Discula oxytropis								
Discula cheiranticola								
Discula calcigena								3
Discula pulvinata								
Discula attrita	11		12		2	5		1
Discula bulwerii								
Discula cockerelli								
Discula tectiformis	3	6		14			28	38

(continues)

(continued)

A/I Species/Samples	0.806 10	0.754 3	0.754* 5	0.715* 9	0.663* 4	0.573 8	0.349 1	0.347 2
Pseudocampylaea portosanctana								
Pseudocampylaea lowei								
Leptaxis sp.								
Leptaxis chrysomela								
Leptaxis cf. *fluctuosa*	4	5		17	18	1		4
Leptaxis nivosa	29	18	2	6	2		13	22
Leptaxis wollastoni								
Lampadia webbiana								
Helix subplicata			3					
Theba pisana								
No. of taxa	16	12	16	12	14	11	10	13
No. of shells	413	199	294	158	179	107	169	185

APPENDIX 4

Taxa recorded at Site 1, samples ordered by A/I ratio and stratigraphical position. Asterisked A/I values indicate high variability within samples (Appendix 1); + = an approximate number. See Fig. 3.

A/I Species/Samples	0.806 10	0.754 3	0.754* 5	0.715* 9	0.663* 4	0.573 8	0.349 1	0.347 2	0.19 6	0.167 7
Craspedopoma mucronatum	1				2					
Truncatellina linearis		1								
Staurodon saxicola										
Leiostyla subcorneocostata										
Leiostyla calathiscus										
Leiostyla monticola		1								
Phenacolimax portosanctanus										
Amphorella melampoides										
Amphorella triticea		11	42	1		19		1	1	2
Amphorella oryza				5						
Amphorella gracilis										
Cylichnidia ovuliformis										
Boettgeria lowei										
Heterostoma paupercula		5	166	9		7		2	21	12
Geomitra coronata		1	90	10	4	3		2	1	7
Spirorbula obtecta	16		11	1		16			3	
Spirorbula depauperata	3	15	50	5	1	14			3	

(continues)

(continued)

A/I	0.806	0.754	0.754*	0.715*	0.663*	0.573	0.349	0.347	0.19	0.167
Species/Samples	10	3	5	9	4	8	1	2	6	7
Caseolus compactus (large)	30			2		2		11		
Caseolus compactus (small)	24	31	300+	29	16	32	1	12	14	97
Caseolus cf. *compactus vigiae*		1	4	24		27	1	9	7	
Caseolus consors									3	
Caseolus abjectus agg.	39	7	26	8	1	8				
Caseolus subcalliferus submajor		5	5	12	4			5	21	12
Caseolus s. subcalliferus						1		5		
Caseolus hartungi										
Caseolus punctulatus	15	6	15	14	4	30	8	8	45	20
Caseolus bowdichianus	20	6	15	10	15	5	33	13	15	12
Actinella arcinella pap.		5	4	4		6				
Actinella crassiuscula	7	11	2	1						
Actinella effugiens										
Discula rotula										
Discula bicarinata										
Discula bicarinata aucta										
Discula oxytropis										
Discula cheiranticola									1	
Discula calcigena										
Discula pulvinata										
Discula attrita			3	3	3	3	1	3	5	3
Discula bulwerii										
Discula cockerelli										
Discula tectiformis		4	2	5	3	8	21		7	4
Pseudocampylaea portosanctana	20									
Pseudocampylaea lowei	4									
Leptaxis sp.										
Leptaxis chrysomela						2			28	3
Leptaxis cf. *fluctuosa*	1	53	5	31	1		10			
Leptaxis nivosa	21	14	8	21	6	5		10	36	5
Leptaxis wollastoni										
Lampadia webbiana										
Helix subplicata		1	5	2						
Theba pisana										
No. of taxa	13	18	18	20	12	17	7	12	16	11
No. of shells	201	178	753	197	60	188	75	81	211	177

APPENDIX 5

Taxa recorded at Site 2, samples arranged by A/I value and stratigraphical position. Samples for which only a few specimens were removed for A/I analysis are omitted.

A/I	0.63	0.59	0.58	0.51	0.46	0.43	n/a	0.42	0.35	0.33	0.13	0.13	0.05	0.03	n/a	0.02
Species/Samples	15	16	7	6	1	12	17	14	13	11	18	10	3	5	9	4
Craspedopoma mucronatum																
Truncatellina linearis																
Staurodon saxicola																
Leiostyla subcorneocostata																
Leiostyla calathiscus																
Leiostyla monticola		2														
Phenacolimax portosanctanus		1	1				1	1	1			1				
Amphorella melampoides	2															
Amphorella triticea	5	42	2			1		3	3	5	1				5	
Amphorella oryza					3											
Amphorella gracilis												1				
Cylichnidia ovuliformis					1											
Boettgeria lowei	1	3	1		11	17		2	8	3						
Heterostoma paupercula	4	41	1		8	4	8	5	5	3	19	13	3	10	14	7
Geomitra coronata	7	29	3		12	7	9	18	5	21	27	12		2	27	1
Spirorbula obtecta	2	9			4	1	13	8	4	7	4	8	2		5	1
Spirorbula depauperata	9	38	4		7	5	8	24	9	21	12	10	5	5	24	
Caseolus compactus (large)																
Caseolus compactus (small)														31		2
Caseolus compactus vigiae	39		8	12	21	20	36	24	11	25	63	45	2	5	31	
Caseolus consors	11	1	5		2	5	27	10	3	11	21	3	2		13	
Caseolus abjectus agg.	1	28			7	13	4	2	6	4	3	3	1	16	1	3

(continues)

(continued)

A/I	0.63	0.59	0.58	0.51	0.46	0.43	n/a	0.42	0.35	0.33	0.13	0.13	0.05	0.03	n/a	0.02
Species/Samples	15	16	7	6	1	12	17	14	13	11	18	10	3	5	9	4
Caseolus subcalliferus submajor		7	2	90	7	7	11	8	2		1	9	3	3	10	
Caseolus subcalliferus subcalliferus										2					1	
Caseolus hartungi																
Caseolus punctulatus	12	7	10	55	35	25	17	8	3	2	2	14	3	15	8	21
Caseolus bowdichianus	5	3	8		25	2	20	17	5	1	7	9	3	6	10	5
Actinella arcinella pap.	2	4		1			4	4		1		8	1		2	1
Actinella crassiuscula																
Actinella effugiens	6	9			15	2		4	4	2		1	5	10	3	2
Discula rotula								3	5	3		2			12	
Discula bicarinata															1	
Discula bicarinata aucta	3	20														
Discula oxytropis					17	11		10	5							
Discula cheiranticola			1	25		12			5							
Discula calcigena																
Discula pulvinata	3	2			14		15			1						
Discula attrita																
Discula bulwerii																
Discula cockerelli																
Discula tectiformis	10	9	4	4	10	2	12	4	3	4	3	9	6	2	7	3
Pseudocampylaea portosanctana	15	8			7	3	1	4	4					5		12
Pseudocampylaea lowei										14	7				2	
Leptaxis sp.																
Leptaxis chrysomela							7									
Leptaxis cf. *fluctuosa*		2		2	3	2		2	1			1			5	
Leptaxis nivosa	6	6	6		43	17	7	22	15	7	2	27	4	3	13	4
Leptaxis wollastoni	2	8	3		7	3	1	19	7					1		
Lampadia webbiana	2								3							
Helix subplicata																
Theba pisana														1	5	11
No. of taxa	21	22	15	7	21	20	17	22	23	19	14	18	13	15	21	13
No. of shells	147	279	59	189	259	159	201	202	117	137	172	176	40	115	199	73

APPENDIX 6

Taxa recorded at Site 12, and at Site 10 (included in the sequence), samples arranged by A/I ratios.

A/I	0.512	0.504	0.442	0.431	0.395	0.329
Species/Samples	12-4	12-3	10	12-2	12-1	12-5
Craspedopoma mucronatum						
Truncatellina linearis						1
Staurodon saxicola					1	
Leiostyla subcorneocostata						
Leiostyla calathiscus						7
Leiostyla monticola					2	2
Phenacolimax portosanctanus						
Amphorella melampoides	15	8	6		15	
Amphorella triticea				14	28	15
Amphorella oryza	4	5				7
Amphorella gracilis						
Cylichnidia ovuliformis						3
Boettgeria lowei			2		6	1
Heterostoma paupercula		6	2	3		13
Geomitra coronata	10	12	4	31	50	4
Spirorbula obtecta	4	7	2	2	1	4
Spirorbula depauperata	13	23	10	14	25	14
Caseolus compactus (large)						
Caseolus compactus (small)						13
Caseolus compactus vigiae				2		
Caseolus consors	2		2	19	54	19
Caseolus abjectus agg.	4	20		6	12	19
Caseolus subcalliferus submajor						
Caseolus subcalliferus subcalliferus					1	1
Caseolus hartungi						
Caseolus punctulatus	15	15	5	4	6	8
Caseolus bowdichianus	9	2				
Actinella arcinella pap.		1		1		
Actinella crassiuscula						
Actinella effugiens	43	12		8	5	13
Discula rotula						
Discula bicarinata						
Discula bicarinata aucta						
Discula oxytropis	5	21	4	10	34	5
Discula cheiranticola						
Discula calcigena	4		3			7
Discula pulvinata						
Discula attrita						
Discula bulwerii		5		4	6	2
Discula cockerelli	7					
Discula tectiformis		1			3	
Pseudocampylaea portosanctana	12	9	7		2	2
Pseudocampylaea lowei				4	1	2
Leptaxis sp.						
Leptaxis chrysomela						
Leptaxis cf. *fluctuosa*						1

(continues)

(continued)

A/I Species/Samples	0.512 12-4	0.504 12-3	0.442 10	0.431 12-2	0.395 12-1	0.329 12-5
Leptaxis nivosa	20	12	5	1		
Leptaxis wollastoni	13	2	1			1
Lampadia webbiana						
Helix subplicata	5	2		5	3	
Theba pisana						
No. of taxa	17	18	13	16	19	24
No. of shells	185	163	53	128	255	164

APPENDIX 7

Taxa recorded by MBS at Fonte da Areia, site S3 (equivalent to Site 1 in the GAG series). Sites are arranged in stratigraphical sequence; no A/I determinations are available. Numbering follows original MBS field notes. *C. abjectus** = Identified as *C. commixtus*; *L. fluctuosa*** = rather *L. erubescens*-like. All "small" *C. compactus* were *C. c. ariensis.* This is not true for other sequences, and the distinction made between large and small elsewhere may not apply here. See Fig. 3 for the alignment of this series with Site 1. Note that *Caseolus calculus,* recorded only here, is not listed in any other appendix.

Stratigraphical sequence	oldest										youngest
Species/Samples	27	26.35	26.21	26.9	26.1	28.2	28.1	29.1	30.1	29.17	25.1
Craspedopoma mucronatum						5			2		
Truncatellina linearis	8						14				
Staurodon saxicola							2				
Leiostyla subcorneocostata								1			
Leiostyla calathiscus	1					3		1	3		
Leiostyla monticola							3				
Phenacolimax portosanctanus								2			
Amphorella melampoides											
Amphorella triticea	2					6	4		8	12	7
Amphorella oryza						1	1				
Amphorella gracilis											
Cylichnidia ovuliformis											
Boettgeria lowei											
Heterostoma paupercula	74	6	5	2		7	8		4	6	69
Geomitra coronata	56	6	5	2		4	11	1	23	22	9
Spirorbula obtecta	10	3	1				1		10	5	
Spirorbula depauperata	50	7	5	4		9	26		6	25	7
Caseolus compactus (large)	266	78	41	29	25	49	83	3	67	26	49
Caseolus compactus (small)								50		50	47
Caseolus cf. *compactus vigiae*		1	16	10			14	10	1	18	25
Caseolus consors											
Caseolus abjectus agg.	36					6*	10		46	7	
Caseolus subcalliferus submajor		11			1		10	15		13	2
Caseolus s. subcalliferus			3								
Caseolus hartungi											
Caseolus calculus			10	2	4					2	

(continues)

(continued)

Stratigraphical sequence	oldest										youngest
Species/Samples	27	26.35	26.21	26.9	26.1	28.2	28.1	29.1	30.1	29.17	25.1
Caseolus punctulatus				2		6	1	26	3	4	
Caseolus bowdichianus		9	1		3	1	5	19		2	2
Actinella arcinella pap.	9								8	7	
Actinella crassiuscula											
Actinella effugiens											
Discula rotula											
Discula bicarinata											
Discula bicarinata aucta									3		
Discula oxytropis											
Discula cheiranticola											
Discula calcigena								2			
Discula pulvinata											1
Discula attrita			1	1	15				3	2	
Discula bulwerii											
Discula cockerelli											
Discula tectiformis	1				6		2	45		2	12
Pseudocampylaea portosanctana											
Pseudocampylaea lowei											
Leptaxis sp.											
Leptaxis chrysomela	1					1	1	19		5	3
Leptaxis cf. *fluctuosa*									1**		
Leptaxis nivosa	2	3	2	1	1	2	5	3	2	7	
Leptaxis wollastoni											
Lampadia webbiana											
Helix subplicata	5						1				
Theba pisana											
No. of taxa	14	9	11	9	7	13	19	14	16	18	12
No. of shells	525	124	90	53	55	100	202	197	190	215	233

APPENDIX 8

Taxa in samples made by MBS at Sites S2 and S1, samples arranged in ascending stratigraphical position in each site. No A/I data are available. Numbers in bold refer to species unique to Site S2, and which are not listed in any other appendix.

	Porto da Morena S2			Ponta da Canaveira S1			
Species/Samples	3	2	1	4	3	2	1
Craspedopoma mucronatum	1		52				
Truncatellina linearis		21	185	26			
Staurodon saxicola			7				
Leiostyla subcorneocostata		3	30	2			
Leiostyla espigaoensis		**4**	**152**				
Leiostyla calathiscus							
Leiostyla monticola							
Punctum pygmaeum		**1**	**42**				

(continues)

(continued)

	Porto da Morena S2			Ponta da Canaveira S1			
Species/Samples	3	2	1	4	3	2	1
Playgyrona placida		**2**	**1**				
Phenacolimax crassus	**1**						
Phenacolimax portosanctanus			4				
Amphorella melampoides			1				
Amphorella triticea	3	5	181			2	
Amphorella oryza							
Amphorella gracilis							
Amphorella cf. *cimensis*			**2**				
Cylichnidia ovuliformis							
Boettgeria lowei				4			
Heterostoma paupercula	2	4	121	44	6	9	36
Geomitra coronata		1	113	22		3	1
Geomitra acarinata		**3**	**106**				
Spirorbula obtecta	1		12	3	6	1	
Spirorbula depauperata	5	6	96	9		3	1
Caseolus compactus (large)							9
Caseolus compactus (small)	11	2	173	45	10	10	19
Caseolus cf. *compactus vigiae*							
Caseolus consors				2			31
Caseolus abjectus agg.	2	1	82	68	1	22	
Caseolus subcalliferus submajor				10	9	2	
Caseolus s. subcalliferus							
Caseolus hartungi			1				
Caseolus punctulatus	2	9	21	27	6	11	25
Caseolus bowdichianus				1			
Actinella arcinella pap.				6			
Actinella crassiuscula							
Actinella effugiens	1		29	11	5	5	
Actinella morenensis		**4**	**130**				
Discula rotula							
Discula bicarinata							
Discula bicarinata aucta				11		1	
Discula oxytropis							
Discula cheiranticola							
Discula calcigena	23		5				
Discula pulvinata					3		84
Discula attrita				1		1	
Discula bulwerii							
Discula cockerelli							
Discula tectiformis					27	1	12
Pseudocampylaea portosanctana							
Pseudocampylaea lowei			11				
Leptaxis sp.		20	4				
Leptaxis chrysomela							
Leptaxis cf. *fluctuosa*	1	11	23			1	3
Leptaxis nivosa	5		6	1	19	1	17
Leptaxis wollastoni							
Helix subplicata				1			
Theba pisana		1					
No. of taxa	13	17	27	19	10	15	11
No. of shells	58	98	1707	294	92	73	238

APPENDIX 9

Taxa in GAG sites with only one or two samples, arranged by A/I ratio or stratigraphy within sites when possible. Site 8: Ilheu do Baixo; Site 3: Ponta da Calheta; Site 11: Ilheu de Ferro; Site 4: São Sebastião; Site 5: Barbara Gomez.
* = including one aberrant, very tall shell, possibly an unrecognised species. Site 4-2 is stratigraphically above 4-1, and 5-1 is above 5-2.

A/I	0.533	0.362	0.517	n/a	n/a	0.639	n/a	0.611	n/a
Species/Sites/Samples	8-1	8-2	3-1	11-1	9-1	4-1	4-2	5-2	5-1
Craspedopoma mucronatum									
Truncatellina linearis									
Staurodon saxicola									
Leiostyla subcorneocostata									
Leiostyla calathiscus									
Leiostyla monticola									
Phenacolimax portosanctanus									
Amphorella melampoides									
Amphorella triticea			1					2	
Amphorella oryza									
Amphorella gracilis									
Cylichnidia ovuliformis									
Boettgeria lowei									
Heterostoma paupercula	9	1	7		27			1	13
Geomitra coronata		4	2					3	
Spirorbula obtecta	3		3	3	2			2	4
Spirorbula depauperata		10	7	15			1	6	2
Caseolus compactus(large)	9	6				4		1	4
Caseolus compactus (small)		36	13			13	6	4	12
Caseolus compactus vigiae									2
Caseolus consors									
Caseolus abjectus agg.	6	6			6			4	
Caseolus subcalliferus submajor		38*	8			4		7	
Caseolus s. subcalliferus									1
Caseolus hartungi	2				5				
Caseolus punctulatus	27	11	12	16	6	62	10	26	4
Caseolus bowdichianus	4	2	14				25	6	10
Actinella arcinella pap.		11	1						1
Actinella crassiuscula									
Actinella effugiens		3		10					
Discula rotula									
Discula bicarinata									
Discula bicarinata aucta									
Discula oxytropis									
Discula cheiranticola		13							
Discula calcigena		7		11	8				1
Discula pulvinata							10		1
Discula attrita	6	4	1			4			
Discula bulwerii									
Discula cockerelli									
Discula tectiformis		3	8			3	25	3	1

(continues)

(continued)

A/I	0.533	0.362	0.517	n/a	n/a	0.639	n/a	0.611	n/a
Species/Sites/Samples	8-1	8-2	3-1	11-1	9-1	4-1	4-2	5-2	5-1
Pseudocampylaea portosanctana					3				
Pseudocampylaea lowei					4				
Leptaxis sp.						3			
Leptaxis chrysomela									
Leptaxis cf. *fluctuosa*		12				3	14	6	2
Leptaxis nivosa		9	7	12			5	25	4
Leptaxis wollastoni									
Lampadia webbiana									
Helix subplicata			8						1
Theba pisana					21				44
No. of taxa	8	17	14	6	9	8	8	14	17
No. of shells	66	176	92	67	82	96	96	96	107

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PUTATIVE NITROXIDERGIC CELLS IN THE DIGESTIVE SYSTEM OF SOME MYTILIDS (MOLLUSCA: BIVALVIA: MYTILIDAE) REVEALED BY NADPH-DIAPHORASE HISTOCHEMISTRY

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ABSTRACT

Distribution and morphology of putative nitroxidergic (NO-ergic) cells were studied in the labial palps, esophagus, stomach, digestive gland, and intestine of the bivalves *Crenomytilus grayanus*, *Modiolus modiolus*, and *Mytilus coruscus* using NADPH-histochemistry (Hope & Vincent, 1989). NO-producing elements were found in all examined organs and regions of the digestive system. NADPH-diaphorase-(d)-positive staining was readily detectable in neurons, secretory cells, brush border epithelial cells, and in the nervous plexuses. Intraepithelial nerve cells were the most common NADPH-d-positive cell type throughout the digestive tube. These cells had a fusiform perikaryon, which gave rise to a thin apical process that extended toward the gut lumen, whereas the basal process contacted the basiepithelial NADPH-d-positive plexus. In the major typhlosole of *C. grayanus*, these cells constituted up to 1.28% of the total number of epithelial cells. The bivalve species studied exhibit a similar distribution pattern of NADPH-d-positive cells, which lie separately or form small groups of two to three in the basal part of the epithelium, being most abundant in the dorsal and ventral regions. Subepithelial NADPH-d-positive neurons were found in *C. grayanus* and *M. modiolus.* The labial palps, lips, and esophagus contained an abundance of intraepithelial and subepithelial secretory cells that stained positive for NADPH-diaphorase. Moreover, NADPH-diaphorase was detected in brush border epithelial cells of the primary and secondary ducts of the digestive gland. All examined regions of the digestive system contained basiepithelial and subepithelial NADPH-d-positive nerve fibers that formed loose to highly developed plexuses. The most prominent plexuses were found in the lips, esophagus, and intestine of the species studied.

Key words: NADPH-diaphorase, nitric oxide (NO) synthase, enteric nervous system, neurons, nervous plexuses, secretory cells, brush border epithelial cells, bivalve.

INTRODUCTION

To date, nitric oxide has been reported to be enzymatically produced within the cell and appears to be involved in the control of cellular and tissue metabolism both in vertebrates and invertebrates. The role of NO in digestive physiology and distribution of NO-producing enzymes in the alimentary canal has been extensively studied in higher (Schleiffer & Raul, 1995; Shah et al., 2004) and lower vertebrates (Olsson & Karila, 1997; Lamanna et al., 1999). On the other hand, our knowledge of the distribution and function of nitric oxide in feeding and digestion of invertebrates is extremely poor. NADPH-diaphorase, an indicator of nitric oxide synthase activity, was detected in the pharynx of the freshwater planarian *Dugesia tigrina* (Esiksson, 1996), trematode *Fasciolopsis buski* (Tandon et al., 2001). Selective histochemical staining was also present in the enteric nervous system of the nematode *Ascaris suum* (Bascal et al., 1995) and the snail *Helix lucorum* (Röszer et al., 2005). NO-ergic cells were also found in the cardiac stomach of asteroids (Martinez et al., 1994; Elphick & Melarange, 1998) and esophagus of gastropods, where NO was shown to act as a sensory neuromodulator (Moroz & Gillette, 1996; Hurst et al., 1999). The role of nitric oxide in the induction and control of feeding response was shown in the *Hydra vulgaris,* which possesses a very primitive olfactory-like system (Colasanti et al., 1997). NO-synthase activity was detected in the centers that control the feeding rhythms, for example, in the buccal ganglia of *Lymnaea stagnalis* (Kobayashi et al., 2000) and *Clione limacina* (Moroz et al., 2000). Nitric oxide was revealed in the epithelial cell of the salivary glands in the blood-sucking bug *Rhodnius prolixus*, where NO was involved in the control of secretion of nitrophorins (Nussenzveig et al., 1995). Moreover, nitric oxide seems to play an important role in feeding and digestion in bivalves; however, the available information on the distribution and function of NO in bivalves is restricted to our reports (Pimenova et al., 2002; Varaksin et al., 2002; Pimenova & Varaksin, 2003).

The present paper focuses on the distribution and morphology of putative nitroxidergic cells and plexuses in all regions of the digestive system in the bivalve mollusks *Crenomytilus grayanus*, *Modiolus modiolus*, and *Mytilus coruscus* (Mytilidae). Elsewhere, we have also reported that nitroxidergic cells occur in the intestine of *C. grayanus* (Varaksin et al., 2002) and *Modiolus kurilensis* (Pimenova et al., 2002), as well as in the labial palps, lips, and esophagus of *Crenomytilus grayanus* (Pimenova & Varaksin, 2003). Our paper combines these scattered data with new original findings to present a general concept of distribution and morphological types of nitroxidergic cells in the digestive system of the most abundant mytilids of the Sea of Japan.

MATERIALS AND METHODS

The study was carried out on mature male and female individuals of the marine bivalves *Crenomytilus grayanus*, *Modiolus modiolus*, and *Mytilus coruscus*, which were collected from Amurskii and Ussuriiskii Bays (Sea of Japan) in the fall 1998–2002. The animals were allowed a two to three days adaptation period, during which they were kept in aquaria with aerated seawater and received no food.

Morphological Methods

For routine morphological examination, portions of the labial palps, lips, esophagus, stomach, digestive gland, and intestine were fixed in Bouin's fluid and/or in 4% paraformaldehyde. Paraffin and/or cryostat (20 µm thick) sections were stained with Ehrlich's hematoxylin and eosin following the generally accepted protocols (Merkulov, 1969).

NADPH-Diaphorase Histochemistry

NO-ergic cells were visualized using NADPH-diaphorase histochemistry (Hope & Vincent, 1989). Positive staining for NADPH-diaphorase has been widely used as a marker for NO-synthase both in vertebrates (Hope et al., 1991; Lamanna et al., 1999) and invertebrates, including mollusks (Moroz & Gillette, 1996; Newcomb & Watson, 2001). The technique is based on the NADPH-dependent conversion of nitroblue tetrazolium, an exogenous substrate, to insoluble diformazan deposits.

Portions of the labial palps, lips, esophagus, stomach, digestive gland, midgut, and hindgut were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h at 4°C. After fixation, the specimens were rinsed in three to four changes of 15% sucrose in 0.15 M Tris-HCl buffer (pH 8.0) for 20 h at the same temperature. Cryostat sections were cut at a thickness of 20 and 40 µm. Complete series of longitudinal and transverse sections were incubated in 0.15 M Tris-HCl buffer (pH 8.0) containing 0.5 µM ß-NADPH (Sigma), 0.5 µM nitroblue tetrazolium (Sigma), and 0.3% triton X-100 for 1 h at 37°C. Then they were rinsed in two changes of distilled water, dehydrated through an ethanol series, and mounted in Dammar Resin. The specificity of the staining was evaluated by adding 10 µM N^{ω}-nitro-L-arginine, and by omitting ß-NADPH or nitroblue tetrazolium.

Statistical Analysis

For each portion of the digestive system, the total number of intraepithelial NADPH-diaphorase-positive cells was counted in ten transverse 20 µm thick sections, and the mean number of cells per section was calculated. For the lips, upper, middle and lower esophagus, as well as for the midgut and hindgut, the number of these cells was expressed as a percentage of the total number of epithelial cells.

Cell width and height, as well as the thickness of the basi- and subepithelial plexuses were measured with an ocular grid and an object micrometer.

The data were processed with Statistica 5.0 software and MS Office package for Windows 2000. The mean and standard deviation was calculated using the unequal variance paired t-test. The difference was considered significant at $p < 0.05$ confidence level.

The slides were examined and photographed with an Olympus BH2-RFCA (model BHS) light microscope.

TABLE 1. Sizes of intraepithelial NADPH-diaphorase-positive secretory cells and nerve plexuses in the labial pulps of bivalve mollusks (Mytilidae); + indicate that loose networks of NADPH-d-positive nerve fibers were found. 0 indicate that NADPH-d-positive elements are absent.

Species	Ridged side			Smooth side		
	cells, μm		plexuses	cells, μm		plexuses
	width	height		width	height	
Crenomytilus grayanus	6.5 ± 0.2	16.3 ± 0.2	0	7.0 ± 0.2	15.4 ± 0.2	0
Modiolus modiolus	0	0	+	6.6 ± 0.1	13.4 ± 0.2	0
Mytilus coruscus	7.1 ± 0.1	17.8 ± 0.1	0	6.1 ± 0.1	9.7 ± 0.2	0

RESULTS

Labial Palps

The bivalve species examined have two pairs of leaf-like labial palps, which lie on either side of the mouth. The oral surfaces of the palps is deeply folded, whereas the outer surfaces are comparatively smooth. The folded structure of the oral surface is due to conspicuous grooves and ridges, the latter leading down to the mouth.

NADPH-diaphorase labeling was detected in numerous secretory cells. The vacuolated cytoplasm was foamy in appearance; the surface contour of the nucleus was often difficult to discern (Fig. 2A). NADPH-d-positive secretory cells occurred on both surfaces of the labial palps in *C. grayanus* and *M. coruscus* and only on the smooth surface in *M. modiolus.* On the smooth side, only intraepithelial cells stained positive for NADPH-diaphorase, while both intraepithelial and subepithelial cells were observed on the ridges side (Fig. 1A). The intraepithelial cells had a large goblet or oval cell body (Table 1) and a distally distended apical process. These cells were often clustered together and occasionally formed large groups, in contrast to the subepithelial cells, which were usually scattered singly. The subepithelial cells were morphologically similar to the intraepithelial cells; the only difference was that the former were more regularly rounded in shape.

The epithelium of the smooth side of the labial palps of *C. grayanus* also contained rare NADPH-d-positive cells with a homogeneously stained cytoplasm and a completely unstained nucleus (Fig. 2A). These cells seemed to be neurons. Their comparatively small subspherical cell bodies gave rise to two thin processes, one of which extended towards the apical surface of the epithelium, whereas another one ran in the opposite direction. On the ridged side of the labial palps, solitary thin subepithelial NADPH-d-positive fibers occurred only in *M. modiolus* (Table 1). They mostly ran parallel to the long axis of the palp and had varicosities along their length. No fibers were observed on the smooth side of the labial palps.

Lips

The ridged surface of the basal part of the labial palps are continuous with smooth regions that give rise to long narrow lips. The latter fuse together thereby surrounding the mouth opening.

In the lips, diformazan deposits labeled two cell types and plexuses. Intra- and subepithelial NADPH-d-positive cells of the first type were found in all the mytilid species examined (Figs. 1A, 2B). They were morphologically similar to NADPH-d-positive secretory cells of the labial palps and were most abundant in the dorsal and ventral regions of the lips. The largest intraepithelial cells, measuring 11.4 ± 0.2 μm in width by 21.6 ± 0.8 μm in height were found in *C. grayanus*.

NADPH-d-positive cells of the second type were most probably neurons. They were found in the lip epithelium of *C. grayanus* and *M. modiolus* (Table 2). These cells had a spindle-shaped cell body, from which an apical process extended toward the apical surface of the epithelium, while the basal one penetrated into the basal portion of the epithelium. The perinuclear cytoplasm and processes showed clear positive labeling, whereas the nucleus was mostly unstained (Fig. 2C). The cells lay in the basal part of the epithelium, being most abundant in the dorsal and ventral regions. They were either scattered singly or clustered together to form groups of two to three.

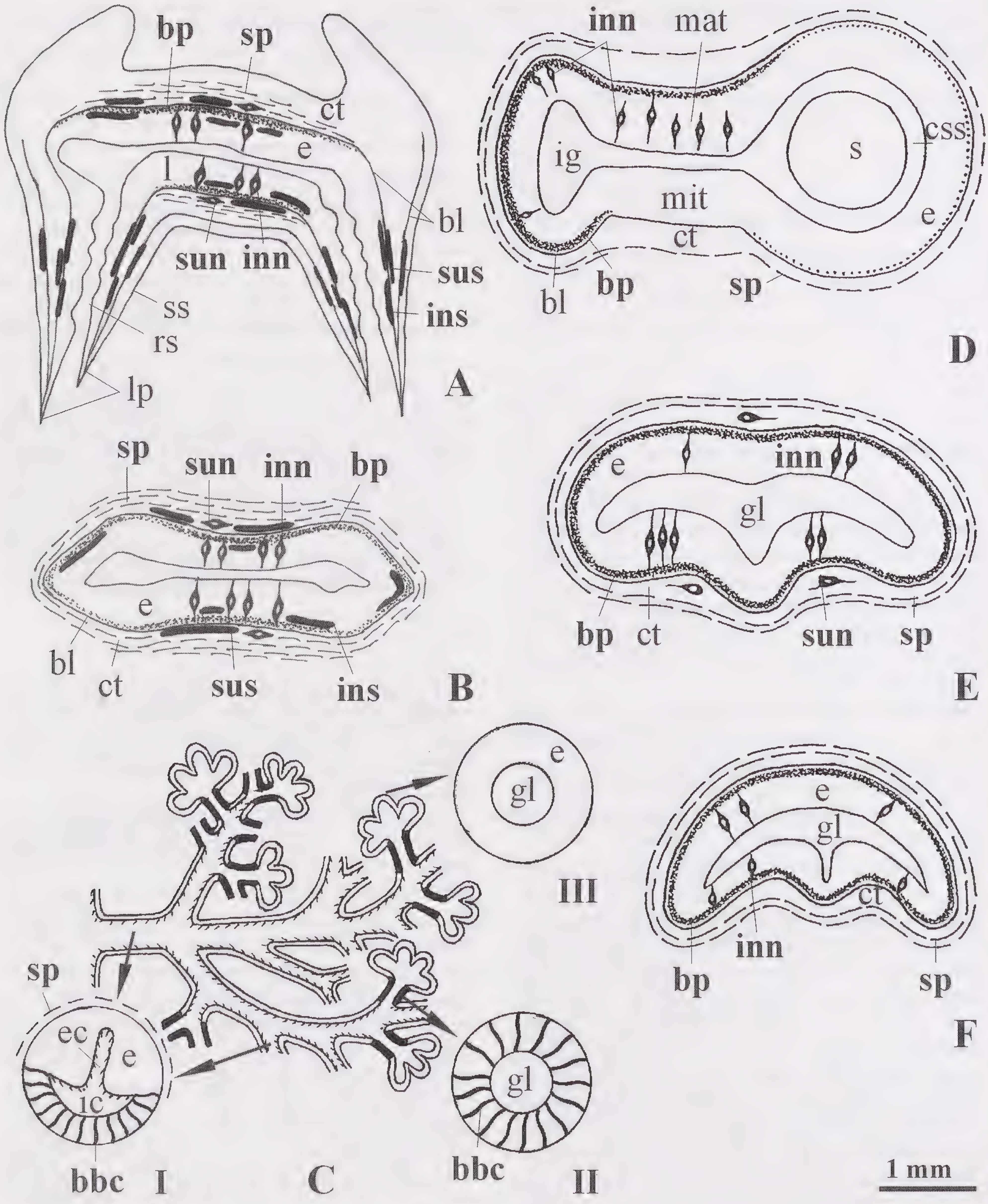

FIG. 1. Schematic drawing showing distribution of NADPH-d-positive elements in the digestive system of *Modiolus modiolus*. A: Labial palps and lips; B: Upper esophagus; C: Digestive gland; D: Anterior midgut; E: Midgut; F: Hindgut. Abbreviations: I, primary duct; II, secondary duct; III, digestive tubule; bbc, brush border cell; bl, basal lamina; bp, basiepithelial plexus; css, crystalline style sac; ct, connective tissue; e, epithelium; ec, excurrent canal; gl, gut lumen; ic, incurrent canal; ig, intestinal groove; inn, intraepithelial nerve cell; ins, intraepithelial secretory cell; l, lip; lp, labial palps; mat, major typhlosole; mit, minor typhlosole; rs, ridged side of the labial palp; s, crystalline style; sp, subepithelial plexus; ss, smooth side of the labial palp; sun, subepithelial nerve cell; sus, subepithelial secretory cell. Note: NADPH-d-positive elements are indicated by bold lettering.

TABLE 2. Major characters of NADPH-d-positive neurons and plexuses in various regions of the digestive tube of bivalve mollusks (Mytilidae); + indicates that rare cells or loose networks of fibers are labeled with diformazan granules. 0 indicates that no positive labeling is observed. Regions of the anterior midgut: CSS, crystalline style sac; IG, intestinal groove; MaT, major typhlosole; MiT, minor typhlosole. *data were previously published in Pimenova & Varaksin (2003); †data were previously published in Varaksin et al. (2002); ‡data were previously published in Pimenova et al. (2002).

Species		NADPH-d-positive cell proportion, %	Cell size, µm		Plexus thickness, µm	
			width	height	basi-epithelial	sub-epithelial
Lips						
*Crenomytilus grayanus**		0.30	3.9 ± 0.1	16.9 ± 0.3	7.9 ± 0.2	71.8 ± 1.4
Modiolus modiolus		0.24	4.7 ± 0.1	17.4 ± 0.3	13.4 ± 0.5	249.1 ± 2.9
Mytilus coruscus		0	0	0	3.7 ± 0.1	27.4 ± 0.5
Upper esophagus						
*Crenomytilus grayanus**		1.01	3.5 ± 0.1	15.1 ± 0.3	7.3 ± 0.2	75.9 ± 1.3
Modiolus modiolus		0.46	4.7 ± 0.1	16.3 ± 0.2	10.9 ± 0.2	181.5 ± 2.7
Mytilus coruscus		0	0	0	5.3 ± 0.2	33.9 ± 0.6
Middle esophagus						
*Crenomytilus grayanus**		0	0	0	+	36.7 ± 0.7
Modiolus modiolus		0	0	0	6.5 ± 0.2	151.3 ± 2.1
Mytilus coruscus		0	0	0	5.5 ± 0.2	41.9 ± 0.6
Lower esophagus						
*Crenomytilus grayanus**		0	0	0	+	33.9 ± 0.8
Modiolus modiolus		0	0	0	+	76.5 ± 1.4
Mytilus coruscus		0	0	0	+	32.1 ± 0.9
Anterior midgut						
Crenomytilus grayanus†	IG	0.51	3.6 ± 0.1	10.9 ±0.2	13.7 ± 0.5	30.1 ± 0.8
	MaT	1.28	5.7 ± 0.2	14.9 ± 0.3	6.7 ± 0.3	31.9 ± 1.1
	MiT	0	0	0	0	12.0 ± 0.7
	CSS	0	0	0	4.2 ± 0.1	5.2 ± 0.1
Modiolus modiolus‡	IG	0.17	2.9 ± 0.1	8.7 ± 0.2	7.5 ± 0.1	26.5 ± 0.3
	MaT	0.20	3.3 ± 0.1	10.8 ± 0.2	5.9 ± 0.2	+
	MiT	0	0	0	0	+
	CSS	0	0	0	8.3 ± 0.2	+
Mytilus coruscus	IG	+	3.9 ± 0.2	10.6 ± 0.3	10.5 ± 0.3	23.6 ± 0.7
	MaT	+	3.4 ± 0.2	13.9 ± 0.3	6.1 ± 0.2	20.7 ± 1.1
	MiT	0	0	0	+	+
	CSS	0	0	0	4.4 ± 0.1	+
Midgut						
Crenomytilus grayanus†		0.83	7.9 ± 0.1	17.3 ± 0.3	11.3 ± 0.2	28.7 ± 0.4
Modiolus modiolus‡		0.14	3.4 ± 0.1	15.5 ± 0.2	14.9 ± 0.1	37.3 ± 0.7
Mytilus coruscus		0	0	0	9.2 ± 0.4	16.1 ± 0.4
Hindgut						
Crenomytilus grayanus†		0.92	5.7 ± 0.1	17.9 ± 0.5	17.3 ± 0.4	66.8 ± 1.2
Modiolus modiolus‡		0.12	3.7 ± 0.1	11.9 ± 0.2	13.5 ± 0.3	57.1 ± 1.9
Mytilus coruscus		0	0	0	10.8 ± 0.3	14.9 ± 0.3

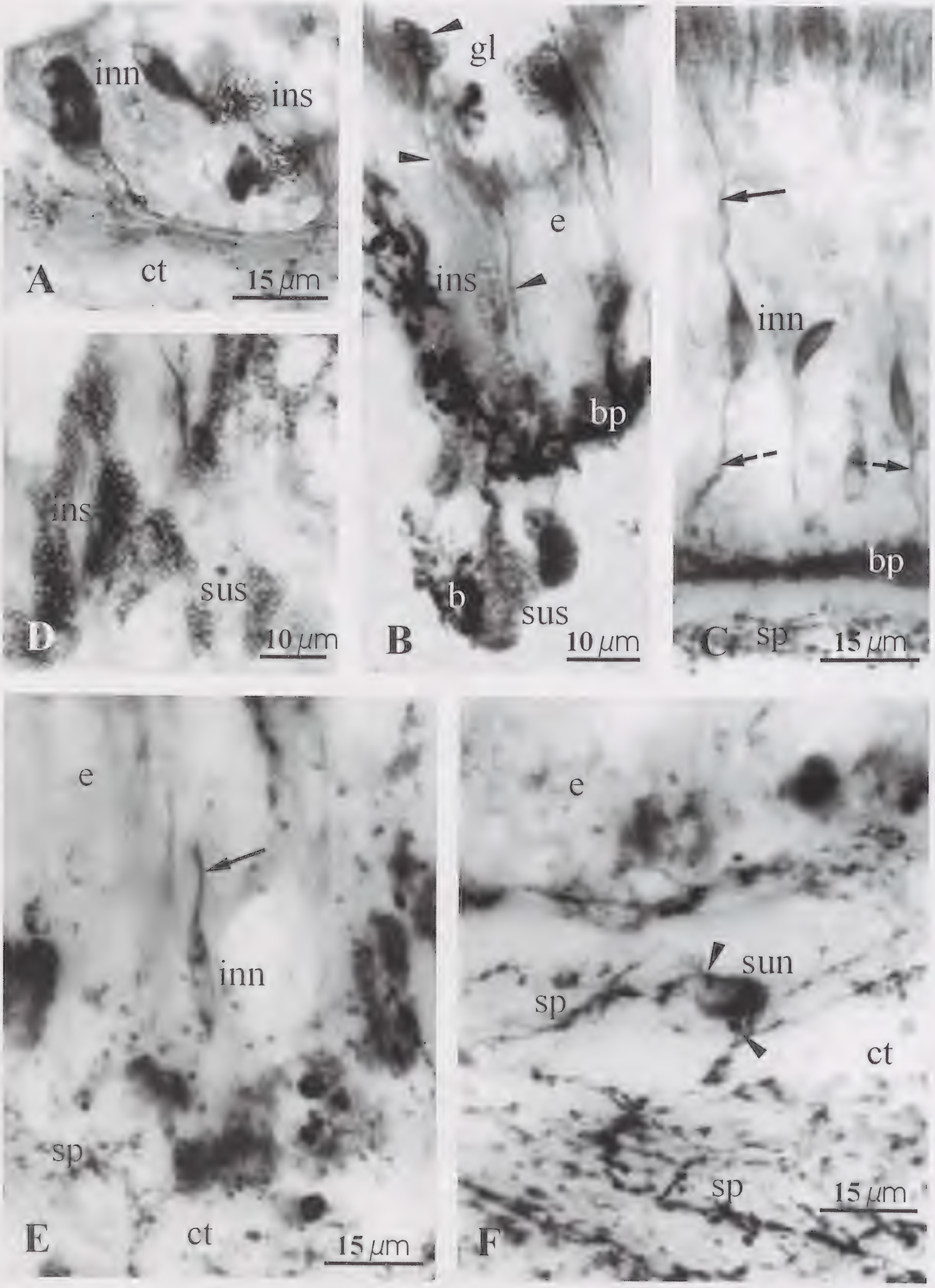

FIG. 2. NADPH-d-positive elements in the labial palps (A), lips (B, C) and upper esophagus (D, E, F) of bivalve mollusks. A: Intraepithelial nerve cells and secretory cell in *Crenomytilus grayanus*; B: Intraepithelial and subepithelial secretory cells in *Modiolus modiolus* (arrowheads show processes of subepithelial cells ending with terminal bulb); C: Intraepithelial nerve cells and plexuses in *Crenomytilus grayanus*; D: Intraepithelial and subepithelial secretory cells in *Mytilus coruscus*; E: Intraepithelial nerve cell in *M. modiolus*; F: Subepithelial nerve cell in *M. modiolus* (arrowhead indicate putative contacts between the neuron and fibers of the subepithelial plexus). Abbreviations: b, transversely cut bundle of nerve fibers; for the other abbreviations, see the caption to Fig. 1. Solid arrows show apical processes emerging from neuronal perikarya. Dashed arrows indicate basal neuronal processes.

Of the species examined, the most prominent basi- and subepithelial networks of nerve processes were observed in the lips of *M. modiolus* (Table 2). In the basal region of the epithelium, NADPH-d-histochemistry labeled longitudinal nerve fibers (Figs. 2B, C). A network of varicose fibers in the subjacent connective tissue also showed positive staining. In the dorsal and ventral regions of the lips, the labeling visualized subepithelial transverse fibers and thick longitudinal bundles of nerve processes (Figs. 2B, C). One could often see short neural bridges connecting the basiepithelial and subepithelial plexuses.

Esophagus

The slit-like mouth opens into a dorsoventrally flattened upper esophagus. The middle and lower parts of the esophagus pass through the digestive gland and are more regularly rounded in shape in cross section. The esophagus is a relatively short, hollow tube with longitudinal epithelial folds.

Upper Esophagus: NADPH-d-positive secretory cells were found in all the mytilid species examined. These cells were morphologically similar to the corresponding cell type of the lips and labial palps. They were most abundant in the dorsal and ventral regions of the upper esophagus (Fig. 1B) and lay both intraepithelially and beneath the epithelium (Fig. 2D). The intraepithelial cells were goblet-shaped, measuring 11.4 ± 0.2 µm in width and 21.6 ± 0.8 µm in height in *C. grayanus*. The nucleus was located close to the basal lamina, whereas the secretory process extended toward the gut lumen and ended with a terminal bulb. The subepithelial cells were more regularly rounded in shape.

NADPH-d-positive nerve cells occurred in the epithelium of *C. grayanus* and *M. modiolus*. They had an elongated fusiform cell body and a thin apical process that ran toward the gut lumen (Fig. 2E). These cells were most abundant in the basal part of the epithelium of the dorsal and ventral regions of the upper esophagus (Fig. 1B). In *C. grayanus*, these cells constituted 1.01% of the total number of epithelial cells (Table 2).

In all species studied, the esophageal epithelium possessed a basiepithelial plexus composed of tightly packed longitudinal NADPH-d-positive fibers (Figs. 2E, F) arranged parallel to one another and showed varicosities of varying shape and size. Of the bivalves studied, the plexus was most highly developed in *M. modiolus* (10.9 ± 0.2 µm thick) (Table 2). There was a direct connection between the basiepithelial plexus and the subepithelial plexus via thin neural bridges or solitary fibers. The subepithelial NADPH-d-positive plexus was composed of thin to thick transverse varicose fibers (Fig. 2F). Longitudinal bundles of densely packed fibers ran along the dorsal and ventral side of the upper esophagus. The subepithelial plexus was also most highly developed in *M. modiolus* (Table 2).

The subepithelial plexus of *M. modiolus* and *C. grayanus* contained rare cells that we regarded as putative neurons (Fig. 2F). These cells were subspherical to elliptical, with major and minor diameters averaging 7.6 ± 0.1 and 11.4 ± 0.2 µm in *M. modiolus* and 6.1 ± 0.1 and 11.7 ± 0.1 µm in *C. grayanus*, respectively. The cells were mostly unipolar, but some are bipolar. The perinuclear cytoplasm and proximal portions of the processes were homogeneously labeled with diformazan, but we failed to visualize the distal parts of the processes.

Middle Esophagus: In all mollusks studied, the epithelium of this portion of the digestive tract contained rare, weakly stained secretory cells. They occurred mostly in the lateral regions, lying either separately or in groups of two to three. No NADPH-d-positive neurons were found.

Basi- and subepithelial NADPH-d-positive plexuses were found in all the mytilid species examined, but also were most highly developed in *M. modiolus* (Table 2). The basiepithelial plexus of the middle esophagus consisted of mostly longitudinally arranged NADPH-d-positive fibers (Figs. 3A, B). NADPH-diaphorase histochemistry also labeled numerous longitudinal, transverse, and oblique fibers of varying diameter and staining intensity that constituted the subepithelial plexus (Figs. 3A, B). The connective tissue that underlay the dorsal and ventral regions of the digestive epithelium often contained longitudinal bundles of nerve processes (Fig. 3A). The two plexuses were connected via solitary fibers (Fig. 3B). In *C. grayanus* and *M. modiolus*, diformazan also labeled the perikarya and processes of subepithelial neurons. These were small unipolar cells with a subspherical to elliptical cell body (Fig. 3C) with major and minor diameters of 7.9 ± 0.1 and 12.9 ± 0.2 µm in *M. modiolus* and 6.5 ± 0.1 and 10.2 ± 0.1 µm in *C. grayanus*, respectively.

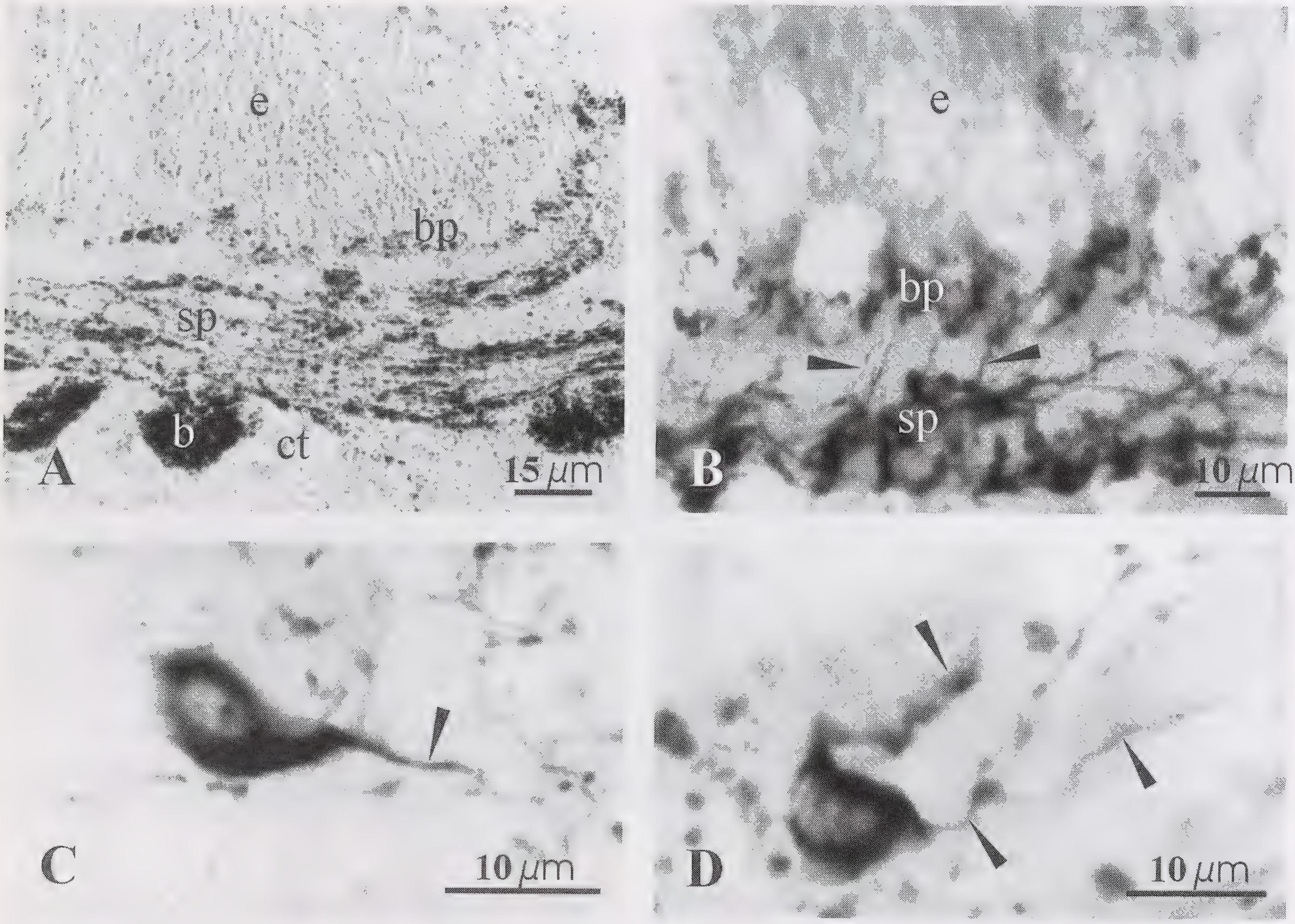

FIG. 3. NADPH-d-positive elements in the middle (A, B, C) and lower (D) esophagus of bivalve mollusks. A: Basiepithelial and subepithelial plexuses in *Crenomytilus grayanus*; B: Connection between the basi- and subepithelial plexuses via solitary fibers (arrowheads) in *Mytilus coruscus*; C: Subepithelial unipolar (arrowhead) neuron in *Modiolus modiolus*; D: Subepithelial bipolar (arrowheads) neuron in *M. modiolus*. Abbreviations are the same as in Figs. 1 and 2.

Lower Esophagus: NADPH-d-positive secretory cells, either scattered singly or clustered together to form small groups, occurred in the lateral regions of the digestive epithelium. Positively stained nerve cells were absent.

The basi- and subepithelial plexuses were composed of fibers of varying diameter and staining intensity (Table 2). The dorsal and ventral regions of the digestive epithelium were underlain by connective tissue that contained thick longitudinal bundles of nerve processes. The two plexuses were interconnected via solitary fibers.

Singly-scattered subepithelial NADPH-d-positive nerve cells occurred in the lower esophagus of *C. grayanus* and *M. modiolus*. These cells had an elliptical cell body that gave rise to one to two processes (Fig. 3D). The perinuclear cytoplasm and processes showed clear positive labeling, while the nucleus remained unstained. The major and minor diameters of the perikarya ranged from 7.8 ± 0.2 and 11.6 ± 0.1 μm (in *M. modiolus*) to 10.2 ± 0.1 and 6.5 ± 0.1 μm (in *C. grayanus*), respectively.

Stomach

The stomach is a sac-shaped organ lined with a heavily ridged mucosa. It is anteriodorsally continuous with the esophagus and leads posterioventrally to the intestine. The stomach is surrounded by the digestive gland, the ducts of which open into the stomach lumen. The anterior portion of the stomach includes a large sorting area with gastric grooves and ridges, and a gastric shield, which is covered by an extracellular plate. The posterior stomach contains a typhlosoles, crystalline style sac, and an intestinal groove.

In all species studied, the heaviest staining was observed in the gastric grooves of the sorting area (Figs. 4A, B). The NADPH-d-positive plexus lay in the basal region of the intestinal groove and consisted of a network of thin longitudinal nerve processes. Singly scattered fibers also ran along either side of the groove. The underlying connective tissue contained rare thin fibers that gave off no branches and ran parallel to the basal surface of the epithelium. Only in *M. coruscus*, NADPH-d-histochemistry labeled rare intraepithelial nerve cells in the basal part of the gastric grooves (Fig. 4B). These cells measured 3.5 ± 0.1 μm in width and 14.1 ± 0.3 μm in height. They either rode upon the basiepithelial plexus or were connected to the latter via their basal processes. The apical process extended toward the gut lumen.

The ridges of the sorting area had no intraepithelial NADPH-d-positive cells and basiepithelial plexus. The underlying connective tissue contained longitudinal bundles of nerve fibers, which, in transverse sections, appeared as either singly-scattered dots or patches of varying morphology and staining intensity.

No NADPH-d-positive elements were found in the gastric shield region. Rare varicose fibers occurred only in the underlying connective tissue.

Of the species examined, only *C. grayanus* possessed intraepithelial nerve cells in the typhlosoles of the posterior stomach (Fig. 4C). They were relatively numerous, and had an elongated fusiform perikaryon and two processes that arose from the opposite poles of the cell body. One of these processes ascended toward the gut lumen, whereas another contacted a moderately developed NADPH-d-positive basiepithelial plexus. These were 3.5 ± 0.03 μm wide and 27.2 ± 0.3 μm high.

In the crystalline style sac and intestinal groove of the posterior stomach of the mytilids, NADPH-d-histochemistry labeled only the basi- and subepithelial plexuses.

Digestive Gland

The digestive gland consists of large primary ducts that extend from the stomach wall and give off short undivided secondary ducts. The latter are continuous with digestive tubules (Fig. 1C).

The lumen of the primary ducts is provided with typhlosole-like longitudinal ridges that serve to separate it into two canals (Fig. 1CI). One the latter is lined with brush border cells and fulfills the functions of an incurrent canal that transports food particles from the stomach to the digestive tubules. The remainder of the lumen functions as an excurrent canal, through which the undigested particles and dead epithelial cells of the tubules pass to the stomach.

In the bivalve species examined in this study, the brush border epithelial cells of the incurrent canal showed homogeneous positive labeling with diformazan (Fig. 4D). These ribbon-shaped cells extended the whole height of the epithelium from the basal lamina to the apical surface and terminated with a small apical bulb (Fig. 4E). The underlying connective tissue contained singly scattered NADPH-d-positive fibers.

In all mytilids, the ciliated epithelial cells of the excurrent canal showed no staining for NADPH-diaphorase (Fig. 4D). NADPH-d-histochemistry labeled a loose subepithelial plexus composed of poorly arborized thin nerve fibers (Fig. 4D).

The secondary ducts were lined with brush border cells (Fig. 1CII), which, like the cells of the incurrent canal, showed positive labeling for NADPH-diaphorase (Fig. 4F). Rare, thin NADPH-d-positive fibers with small varicosities occurred only beneath the epithelium. They were poorly arborized and extended deeply into the underlying connective tissue.

In all species studied, the cells of the digestive tubules exhibited no positive labeling for NADPH-diaphorase (Figs. 1CIII, 4F). The underlying connective tissue contained numerous fibers with heteromorphic varicosities. These fibers were either scattered singly or arranged in thick bundles (Figs. 4D, F).

Intestine

The intestine is conventionally subdivided into the anterior midgut that contains a crystalline style, the midgut proper, and the hindgut.

Anterior Midgut: In mytilids, the anterior midgut is composed of a tube that possesses the intestinal groove, the major and minor typhlosoles, and the crystalline style sac (Fig. 1D). In all mollusks studied, the most intense NADPH-d labeling was observed in the intestinal groove (Fig. 5A). The subpopulation of NAPDH-d-positive intraepithelial neurons ranged from a single cell (in *M. coruscus*) to 0.51% of the total number of epithelial cells (in *C. grayanus*) (Table 2). A thin apical process extended toward the gut lumen. The basal region of the intraepithelial neurons was

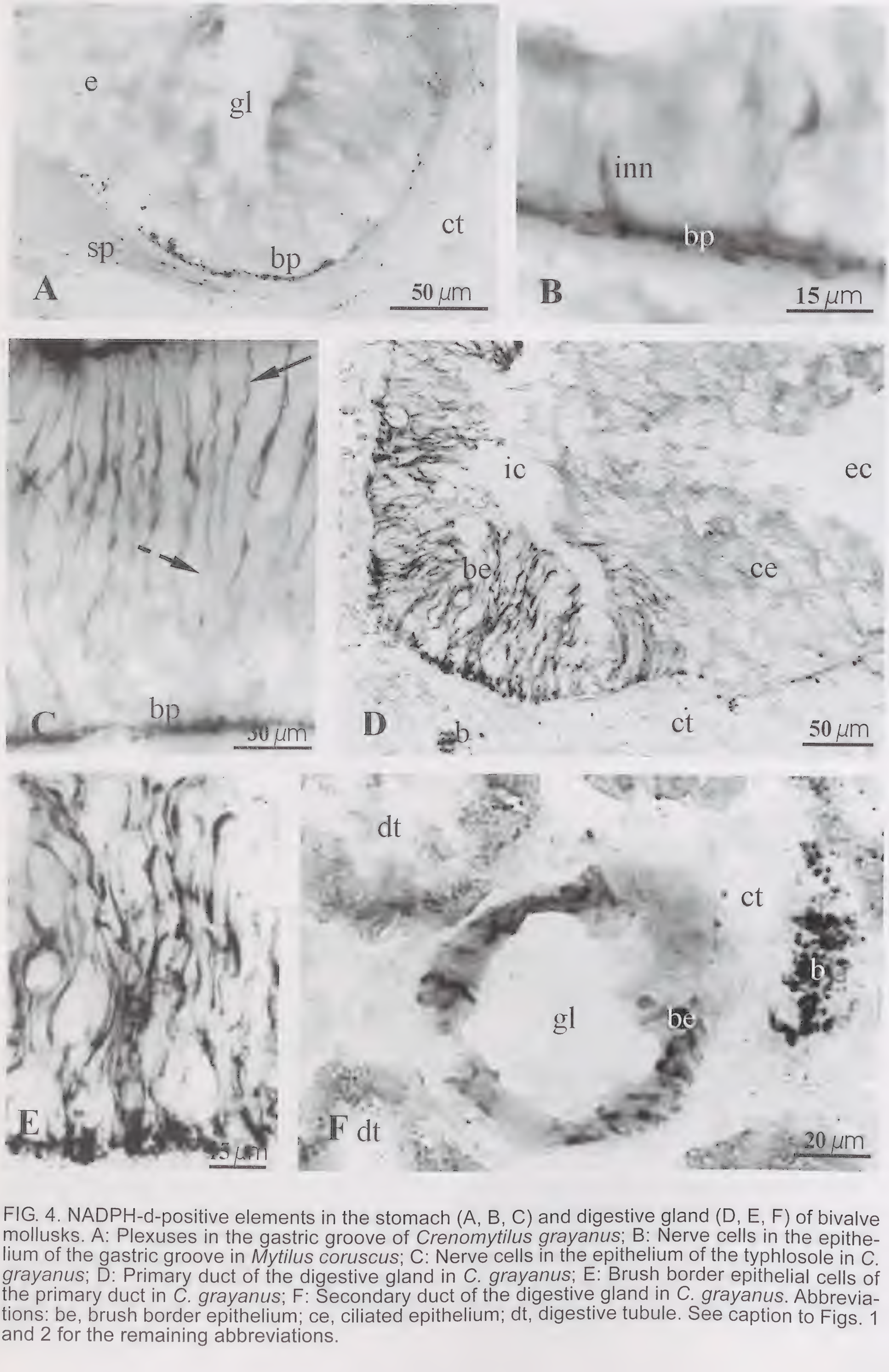

FIG. 4. NADPH-d-positive elements in the stomach (A, B, C) and digestive gland (D, E, F) of bivalve mollusks. A: Plexuses in the gastric groove of *Crenomytilus grayanus*; B: Nerve cells in the epithelium of the gastric groove in *Mytilus coruscus*; C: Nerve cells in the epithelium of the typhlosole in *C. grayanus*; D: Primary duct of the digestive gland in *C. grayanus*; E: Brush border epithelial cells of the primary duct in *C. grayanus*; F: Secondary duct of the digestive gland in *C. grayanus*. Abbreviations: be, brush border epithelium; ce, ciliated epithelium; dt, digestive tubule. See caption to Figs. 1 and 2 for the remaining abbreviations.

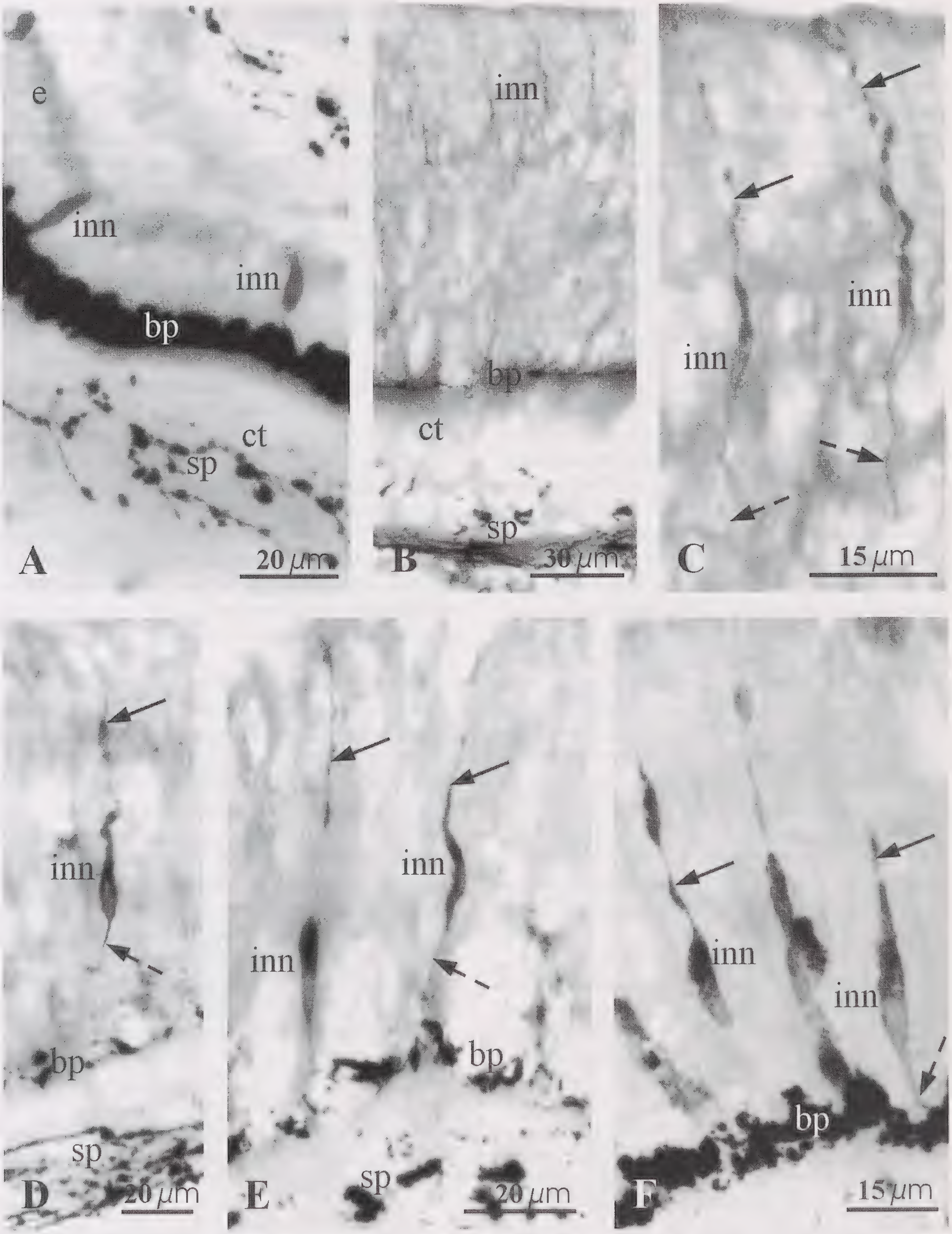

FIG. 5. NADPH-d-positive elements in the intestine of the bivalve mollusks. A: Intraepithelial nerve cells and plexuses in the intestinal groove of *Crenomytilus grayanus*; B: Major typhlosole in the anterior midgut of *C. grayanus*; C: Nerve cells in the epithelium of the major typhlosole in *C. grayanus*; D: Midgut of *Modiolus modiolus*; E: Hindgut of *M. modiolus*; F: Nerve cells and plexus in the epithelium of the hindgut in *C. grayanus*. See caption to Figs. 1 and 2 for abbreviations.

swollen and contributed to the basiepithelial plexus. The latter showed an intense blue staining and was composed of numerous bundles of nerve fibers. The NADPH-d-positive basi- and subepithelial plexuses were interconnected via thin fibers.

The epithelium of the major typhlosole also contained NADPH-d-positive nerve cells, which possessed a fusiform cell body, and were interspersed among other cell type in the apical region of the epithelium (Figs. 5B, C). These cells also occasionally occurred between the intestinal groove and major typhlosole and between the major typhlosole and crystalline style sac. The labeled cells were most abundant (1.28%) in the epithelium of *C. grayanus* (Table 2). In the major typhlosole of the all species studied, the basal region of the epithelium showed a dense network of longitudinal positively stained fibers (Fig. 5B). NADPH-d-histochemistry also labeled cross cut bundles of nerve fibers that lay in the segment, adjacent to the crystalline style sac. The basiepithelial plexus was connected with the NADPH-d-positive subepithelial plexus (Fig. 5B).

No positive labeling for NADPH-diaphorase was observed in the cells of the minor typhlosole. The basiepithelial plexus was also often absent (Fig. 1D); however, rare fibers occurred in the basal portion of the epithelium only in *M. coruscus*. The subepithelial NADPH-d-positive varicose fibers were arranged in loose networks (Table 2).

The epithelium of the crystalline style sac also showed no positive staining for NADPH-diaphorase. The basi- and subepithelial plexuses were composed of loosely spaced fibers (Table 2; Fig. 1D).

Midgut: NADPH-d-positive neurons were found in the gut epithelium of *C. grayanus* and *M. modiolus* (Figs. 1E; 5D). The thin apical process was clearly seen; it ran from the cell body toward the gut lumen. The basal process contributed to the basiepithelial plexus. The intraepithelial neurons constituted 0.14% (in *M. modiolus*) to 0.83% (in *C. grayanus*) of the total number of epithelial cells.

The NADPH-d-positive basiepithelial plexus was composed of longitudinally oriented fibers (Fig. 5D). As in other gut regions, the basi- and subepithelial plexuses were loosely connected via solitary nerve fibers. The subepithelial plexus consisted of densely packed fibers, showed a dark-blue staining (Fig. 5D), and measured 16.1 ± 0.4 μm (in *M. coruscus*) to 37.3 ± 0.7 μm (in *M. modiolus*) thick (Table 2). Only in *M. modiolus*, on the ventral (concave) side of the intestine and occasionally on the dorsal (convex) side, the subepithelial plexus contained NADPH-d-positive nerve cells (Fig. 1E), with the minor and major diameters of 5.2 ± 0.2 and 13.5 ± 0.2 μm, respectively. These were most often unipolar neurons; however, some were bipolar, and the least abundant were multipolar neurons. Their processes either contributed to the subepithelial plexus or extended deeply into the connective tissue.

Hindgut: The hindgut is a hollow tube that, after entering the digestive gland, and passes through the pericardial sac and the ventricle of the heart, proceeds to the posterior adductor muscle, passes over the dorsal surface of the latter to end at the anus.

In the hindgut of *C. grayanus* and *M. modiolus*, NADPH-d-histochemistry stained intraepithelial nerve cells (Table 2; Fig. 1F). These were fusiform cells that were either scattered singly or clustered together to form small groups (Figs. 5E, F). Diformazan homogeneously labeled the perikarya and the processes to their full extent, including the most distal parts, while the nucleus remained unstained.

TABLE 3. Distribution of NADPH-d-positive elements in the digestive system of bivalve mollusks (Mytilidae); ++, intraepithelial NADPH-d-positive neurons and plexuses; +, NADPH-d-positive plexuses; 0, no NADPH-d-positive neurons and plexuses were observed; (+), NADPH-d-positive secretory cells; [+], NADPH-d-positive brush border cells.

Species	Labial palps	Lips, upper esophagus	Middle and lower esophagus	Stomach	Digestive gland	Midgut	Hindgut
Crenomytilus grayanus	0 (+)	++ (+)	+ (+)	++	+ [+]	++	++
Modiolus modiolus	+ (+)	++ (+)	+ (+)	+	+ [+]	++	++
Mytilus coruscus	0 (+)	+ (+)	+ (+)	++	+ [+]	++	+

In all species studied, the NADPH-d-positive basiepithelial plexus consisted of a dense heavily stained network of nerve fibers. The subepithelial plexus comprised varicose fibers that ran in various directions and were often arranged in bundles (Figs. 5E, F).

The data presented above suggest that NADPH-d-positive elements occur in all parts of the bivalve digestive system and include heteromorphic cells and nerve plexuses (Table 3).

No positive labeling for NADPH-diaphorase was observed in the control sections.

DISCUSSION

Nerve Cells and Plexuses

The paper gives an account of distribution and morphology of putative nitroxidergic cells and fibers in the digestive system of *Crenomytilus grayanus*, *Modiolus modiolus*, and *Mytilus coruscus* (Mytilidae). In the bivalves studied, virtually all of the gut regions contain NADPH-d-positive fusiform cells, the thin apical process of which extends toward the gut lumen, while the basal process runs in the opposite direction and feeds into the basiepithelial putative nitroxidergic plexus. In the gut epithelium of many invertebrate taxa, including bivalves, specialized cells were found that are not directly involved in digestion but fulfill regulatory functions. In bivalves, the enteric regulatory system is well developed and seems to comprise both nerve and endocrine cells (Punin, 2001). Electron microscopy studies of the intestinal epithelium in *Arctica islandica* and *Mytilus edulis* revealed cells that were similar to neurons in terms of ultrastructural features (Punin, 1981, 1989). These cells possess a well-developed, rough endoplasmic reticulum, prominent Golgi bodies, various types of granules, and processes that arise from the perikaryon and feed into the basiepithelial plexus. Vital staining with methylene blue visualizes receptor nerve cells in the midgut epithelium of *Anodonta cellensis* (Gilev, 1952). Our results show that the intraepithelial cells in the lips, esophagus, stomach, and intestine of the bivalves examined are similar, both in terms of morphology and distribution, to the cells of the enteric regulatory system of the bivalves studied by other authors (Punin, 1981, 1989, 2001; Gilev, 1952). These cells seem to meet all morphological criteria of nerve cells.

According to reports of a number of authors, the lips, mouth, tentacles, and pneumostome of gastropod mollusks are provided with aggregations of neurosensory cells that give rise to sensory organs in some species (Zylstra, 1972). For instance, receptor cells were found beneath the epidermis of the foot, head, mouth, lips, and tentacles in the pulmonates *Lymnaea stagnalis* and *Helix vulgaris* (Zaitseva, 1980). The author refers to these cells as primary bipolar receptors.

Our results demonstrate that in bivalves studied, virtually all regions of the digestive system contain fibers that stain blue with NADPH-d-histochemistry and lie both in the basal region of the epithelium and in the underlying connective tissue. It has been previously shown that the alimentary canal of bivalves is provided with a complex system of basiepithelial and subepithelial nerve plexuses (Giusti, 1970; Punin, 1981, 1989). Electron microscopy examination shows that the two plexuses differ in size of granules in the nerve processes and, therefore, may contain different biologically active substances. The subepithelial plexus is thought to be a part of the peripheral nervous system (Punin & Konstantinova, 1988; Punin, 1989).

Vital staining with methylene blue and histofluorescence studies suggest that the basiepithelial plexus is composed of processes that arise from the intraepithelial cells (Punin, 1981; Punin & Konstantinova, 1988). However, if we assume that only intraepithelial NO-ergic cells send their nerve processes to the basiepithelial NO-ergic plexus, then it would be difficult to explain why the latter is so well developed in certain cases. This plexus is most probably connected with cells and fibers that lie in the connective tissue. Light and electron microscopy studies on *M. edulis* (Punin & Konstantinova, 1988) and *A. islandica* (Punin, 1981), respectively, demonstrate that nerve processes of the basiepithelial nerve plexus occasionally penetrate into the underlying connective tissue. Moreover, in the hindgut of *Tapes watlingi* (Dougan & McLean, 1970) and midgut of *Mytilus galloprovincialis* (Giusti, 1970), nerve fibers lying in the connective tissue were found to invade the basal region of the epithelium. Consistent with these observations, we often observed nitroxidergic fibers that connected the NO-ergic, basi- and subepithelial plexuses. The above findings suggest that the two plexuses should be referred to as interconnected parts of the anatomically continuous bivalve enteric nervous system.

It is worth noting that in spite of a relatively small proportion of putative NO-ergic cells in the gut epithelium, the basiepithelial plexus is highly developed. We have elsewhere shown that although only relatively a small number of perikarya show positive labeling in ganglia of bivalve mollusks, the neuropile areas contain plenty of NO-ergic fibers (Annikova et al., 2000). A similar pattern of staining was observed in the central nervous system of other bivalve species, as well as in gastropods (Moroz & Gillette, 1996; Dyuizen et al., 1999). A study carried out on the bivalve *C. grayanus* suggests that NO production is not restricted to the cell body; nerve processes are believed to be capable of local synthesis of nitric oxide (Annikova et al., 2000). Consistent with this suggestion, our results showed the localization of NO-synthase in numerous fibers that bear varicosities along their length. Varicose swellings have been commonly regarded as sites where neurotransmitters and other biologically active substances are produced and released.

In *C. grayanus* and *M. modiolus*, the subepithelial plexus contained singly scattered unipolar, bipolar or sometimes multipolar NADPH-d-positive cells. In the esophagus of *A. islandica* and intestine of *M. edulis*, the subepithelial tissues contain a cell type with processes that penetrate into the epithelium to extend toward the gut lumen (Punin, 1981, 2001; Punin & Konstantinova, 1988). Our results showed that the processes of subepithelial NO-ergic cells either terminated within the adjacent tissues or contacted the fibers of the subepithelial plexus rather than invading the epithelium. Gilev (1952) visualized bipolar neurons in the subepithelial plexus of *A. cellensis* using methylene blue staining. The author referred to these cells as receptors generating sensory impulses. In pulmonates, rare nerve cells were found in the subcutaneous plexus and among the distal branches of nerves in the head, mouth, lips, and tentacles (Zaitseva, 1980). Most of these cells are believed to be interneurons that mediate horizontal and vertical connections between receptors and are responsible for interactions between the sensory cells and efferent fibers. These data are consistent with our hypothesis that putative NO-ergic cells in the digestive system of bivalves are nerve cells that are probably involved in transmission of sensory signals to the central nervous system and in preliminary processing of information.

Thus, our results show that the digestive system of bivalve mollusks is provided with a well-developed putative nitroxidergic nervous system, which comprises intra- and subepithelial neurons, basi- and subepithelial plexuses. Putative nitroxidergic cells can act both as receptors and as effectors and, therefore, can be regarded as components of local reflex arcs. The NO-ergic cells seem to be connected to the central nervous system and thereby fulfill functions of a sensory component of regulatory circuits (Balashov et al., 1992; Punin, 2001). These putative receptors might release a biologically active substance in response to chemical stimuli from the gut lumen. The intestinal epithelium of bivalves is known to possess a complex heterogeneous system of regulatory cells, which contain various monoamines and oligopeptides (Punin, 2001). Our studies have shown that, besides other biologically active substances, these cells seem to produce nitric oxide.

Nitric oxide is believed to play a key role in sensory processing mechanisms (particularly, in the olfactory system) in various animal taxa ranging from coelenterates to mammals (Colasanti & Venturini, 1998). Studied carried out on gastropods, the nitroxidergic neurons of which have been extensively studied, also suggest that these cells primarily act as receptors (Moroz et al., 2000; Gelperin et al., 2000, 2001).

Secretory and Brush Border Epithelial Cells

Besides putative nitroxidergic nerve cells and fibers, NADPH-d-histochemistry also labels non-neuronal cells in the anterior regions of the digestive system and in the digestive gland of the species studied. These cells differ from the putative NO-ergic neurons both in morphology and distribution pattern.

In the labial palps, lips, and esophagus, we observed numerous intra- and subepithelial secretory cells that stained positive for NADPH-diaphorase. Frolova (1988) previously showed that, besides predominant ciliated cells, numerous intra- and subepithelial secretory cells occur in the labial pulps, lips, and esophagus of *C. grayanus*. This suggests that in the labial palps, lips, and esophagus of the species studied some of the intra- and subepithelial secretory cells do contain NO-synthase.

NADPH-diaphorase labeling was also observed in brush border cells, but not in ciliated cells, in the luminal epithelium of the primary and secondary ducts of the digestive gland. This labeling seems to be due to the presence of NO-synthase in the brush border cells that line the incurrent canal of the primary and secondary ducts, the major function of which is to transport food particles (Leibson & Usheva, 1979).

NO-synthase was found both in neurons and non-neuronal cell types, including epithelial cells, in a variety of animal taxa and in human tissues (Proskuryakov et al., 1999). For instance, NADPH-d-histochemistry labeled non-neuronal cells in the peripheral olfactory organs of the gastropod *Clione limacina* (Moroz et al., 2000). The authors referred to them as secretory-like cells. In another study, circumoral epithelial tissues of the nudibranch *Melibe leonina* showed positive staining for NO-synthase (Newcomb & Watson, 2001). In the earthworm *Lumbricus terrestris*, NO-synthase immunoreactivity was seen in the mucous cells within the epidermis (Licata et al., 2002).

Possible Functional Significance of Nitric Oxide

Heavy NADPH-diaphorase staining labels the intraepithelial neurons and basiepithelial plexus that make direct contacts with ciliated epithelial cells of the digestive tube. Nitric oxide is believed to be capable of controlling ciliary beating frequency, as was shown for the epithelial cells of the mantle integument of the mussel *M. galloprovincialis* (Licata et al., 2003). Both our data and reports of other authors suggest that nitric oxide regulates ciliary currents that transport food particles and feces along the digestive tube. However, no NO-ergic cells were observed associating with the epithelium of the crystalline style sac, which possessed a well-developed locomotory apparatus. Surprisingly, the basiepithelial plexus is not highly elaborated in this epithelium. This suggests that nitric oxide is not the only substance that affects the ciliary beating in the digestive tract of bivalve mollusks. Similarly, NADPH-d labeling is not evenly distributed throughout the ducts of the digestive gland in the species studied. It is worth noting that only rare positively stained subepithelial fibers occur beneath the ciliated epithelium of the excurrent canal of the primary duct, whereas almost a half of brush border epithelial cells of the incurrent portion of the primary and secondary ducts show specific staining with diformazan granules.

In addition to their main locomotory function, ciliated epithelial cells are also involved in secretion (mucous synthesis, secretion of digestive enzymes and proteinaceous components of the crystalline style), absorption, and deposition of nutrients (Owen, 1966; Reid, 1966; Giusti, 1970). This mostly applies to the cells of the epithelial lining of the lips, esophagus, intestinal groove, major typhlosole, midgut, and hindgut. These regions are provided with NO-ergic neurons and show well-developed basi- and subepithelial plexuses. On the other hand, the minor typhlosole and crystalline style sac have a relatively low secretory activity and, correspondingly, possess poorly developed nitroxidergic plexuses and no nitroxidergic cells. This suggests that nitric oxide is involved in the regulation of secretion and absorption in the gut of bivalve mollusks. This is consistent with our observations of numerous NADPH-d-positive secretory cells in the anterior regions of the digestive system. The role of nitric oxide in the control of secretion and absorption in the mammalian alimentary canal has been extensively studied (Schleiffer & Raul, 1997; Shah et al., 2004). It has been also demonstrated that NO is involved in the regulation of mucous secretion in the mantle integument of *M. galloprovincialis* and epidermis of *L. terrestris* (Licata et al., 2002, 2003).

Thus, our data show that the digestive system of the mytilids *Crenomytilus grayanus*, *Modiolus modiolus*, and *Mytilus coruscus* contains a system of heteromorphous putative NO-ergic cells. NADPH-diaphorase was demonstrated to occur in the intra- and subepithelial nerve cell, as well as in the basi- and subepithelial nerve plexuses in all regions of the digestive tube. Moreover, NADPH-diaphorase was detected in the intra- and subepithelial secretory cells of the labial palps, lips, and esophagus and in the brush border epithelial cells of the primary and secondary ducts of the digestive gland.

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SCHISTOSOMIASIS JAPONICA INTERVENTION STUDY ON POYANG LAKE, CHINA: THE SNAIL'S TALE

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ABSTRACT

A U.S. National Institutes of Health-sponsored study was initiated in 1998, ending in 2002, to examine the hypothesis that bovines, buffaloes in particular, were responsible for the persistence of schistosomiasis in human populations living in and around Poyang Lake, Jiangxi Province, P. R. China. The two villages are the focus of this paper, the experimental Jishan Village and the control Hexi Village. The prevalence and intensity of infection of humans and buffaloes were determined. Then, all inhabitants of all the villages were treated with praziquantel, while only the buffaloes of the experimental (intervention) village were treated. Following treatment, rates of re-infection of people in the two villages and buffaloes in the experimental village, as well as the ongoing prevalence of infections in the buffaloes of the control village were monitored annually.

Quantitative collections of snails were made pre- and post-flood for a total of nine seasons to determine the density of snails, density of infected snails, and prevalence of infected snails. Collections were made from ecologically different village zones and from hot spots for persistent snail infections within zones. The hypothesis was that treatments in the experimental village would drive down snail infections to 80% or more below pre-trial levels. This paper makes the case that the intervention did not achieve reducing snail infections village-wide by even 50% or more. The probable reasons for this failure are discussed.

The significant findings of this longitudinal study are several: (1) Foremost, the intervention did not achieve the goal of significantly reducing snail infections in Jishan. (2) To understand the dynamics of *Schistosoma japonicum* transmission throughout large areas, such as the administrative villages of Hexi and Jishan, it is important to partition the area into clearly definable ecological zones. (3) We have found that the greatest risk of infection is in very small areas within zones that we call hot spots. (4) The most important indicator for risk of infection is the density of infected snails, not the prevalence. (5) There are very significantly more infected snails per area in hot spots than in non-hot spots. (6) Density of infected snails is not correlated with density of snails overall. (7) The sampling strategy of zones, squares, and random selection of 20 4 m^2 cells within squares has enabled robust statistical analyses of snail populations that have a strong negative binomial distribution. Sampling twice a year (before the floods and after the floods) for at least 8 or 9 seasons has provided a time series enabling a robust assessment of trends in the experimental and control villages. Paying close attention to environmental factors as they impact results is critical.

Key words: Schistosomiasis, epidemiology, intervention, China, Poyang Lake, *Oncomelania hupensis*, *Schistosoma japonicum*, snail infections, hot spots, disease transmission, ecological partitioning, analysis of variance.

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INTRODUCTION

A U.S. National Institutes of Health-sponsored study was initiated in 1998, ending in 2002, to examine the hypothesis that bovines, buffaloes in particular, were responsible for the persistence of schistosomiasis in human populations living in and around Poyang Lake, Jiangxi Province, P. R. China. The study initially involved four villages, but after two years it narrowed to two administrative villages, a control village and an experimental village. The two villages are the focus of this paper. The baseline study on the demographic factors and human/bovine infections in the villages was published (Guo et al., 2001). The companion baseline study of the distribution, density and prevalence of infections of snails was published (Davis et al., 2002a).

Just prior to initiating the study, the prevalence and intensity of infection of humans and buffaloes were determined. Then, all inhabitants of all the villages were treated with praziquantel while only the buffaloes of the experimental (intervention) village were treated. Following treatment, rates of reinfection of people in the two villages and buffaloes in the experimental village, as well as the ongoing prevalence of infections in the buffaloes of the control village were monitored annually. The results of the intervention for humans and buffaloes are reported elsewhere (Guo et al., 2006).

Each year, snail density and prevalence of infections were determined from different ecological zones of each village in which a number of 10,000 m^2 squares were positioned. All snails were collected from a random selection of frames from 20 cells selected at random from 100 cells in each square. From the collections, the percent area that had snails was calculated along with density of snails and prevalence of infections. Theoretically, the elimination of infections in people and buffaloes in the experimental village should result, within two years, in the elimination of infections in snails, and the life cycle would be broken. One would expect to see a decline of infections in snails so that at the end of this study, there would be no infected snails, or, at the very least, over 80–90% of the pre-study infections (density of infections) would be eliminated. For this to occur in the experimental village, (1) the infections in buffaloes would have to be maintained at zero; (2) there could be no immigration of infected buffaloes; (3) all persons in the experimental village would have to be treated for infections when they were diagnosed to be positive for infections; (4) there could be no immigration of infected snails from outside the village.

The purpose of this paper is to present the snail data collected over nine seasons (from 1988 to 2001) and make the case that the intervention did not achieve reducing snail infections village-wide by even 50% or more. The probable reasons for this failure are discussed.

Background

Poyang Lake, the largest lake in China (area of 4,647 km^2), is a major endemic area for schistosomiasis in China. The lake environ-

TABLE 1. Basic statistics for the two administrative villages. Prevalence data were from the baseline paper for humans and cattle (Guo et al., 2001). Na = No data provided; * = data were not provided by village but an overall mean for the four villages initially studied.

	Hexi Village (Control)	Jishan Village (Experimental)
Number of people	1,043	1,280
Number buffalo	364	665
Grazing area (km^2)	3.69	12.1
Infection prevalence people (1996 data)	Na	17%
Infection prevalence buffalo (1996 data)	Na	20%
Baseline infection prevalence humans	11%	20%
Baseline infection prevalence buffaloes	14%*	14%*

ment is unique. High dikes surround the lake and habitable islands to contain annual flood waters from the spring monsoons. During the annual floods, when the lake basin fills up like a bathtub, all persons and cattle live behind the dykes. With the receding floods, the marshlands once again emerge, and cattle go out on the marshlands to forage. The flooding season extends from April or May to September to October. At the end of the flood, the lake loses as much as 90% of its water. All infections occur within the lake basin in the marshlands; there are no snails behind the dikes. The vast grasslands are difficult to reach and traverse. Travel there is by small boat, water-buffalo carts, and walking.

Population and coordinate data for the villages are found in Davis et al. (2002a). The most relevant summary statistics are given in Table 1. The villages studied are a small subset of the 1,125 endemic villages around Poyang Lake with a total population of 1,750,000 persons and an average infection prevalence of 15.2% (circa 1996). There is about one water buffalo or cow for every 10 persons or 150–200 cattle per village throughout the marshlands. Overall, in hyper endemic villages, 12.9% of the cattle are infected (Davis et al., 2002a).

METHODS

Methods are given in detail in Davis et al. (2002a, b). The baseline data were collected in late October and November of 1998 (post-flood collection and one following the worst flood in China in 40 year) and early May of 1999 (pre-flood collection). Thus, two collections were made each year (spring or pre-annual flood; fall or post annual flood) for a total of nine seasons (ending Nov. 2002). Remote sensing images (Landsat^{tm}) were used to determine the area of the lake, the area of grazing land, and to differentiate snail habitat from grazing area that was not snail habitat (Davis et al., 1999, 2002a, b; Wu et al., 2002). The positions of all sites were recorded using a GPS (Global Positioning System; Garmen handheld GPS) and all data were managed by GIS (Geographic Information Systems).

The villages were divided into clearly observable different ecological zones (three for Jishan, two for Hexi). The zones were defined by factors of elevation, proximity to human habitation where agriculture was one practiced, and degree of prevailing wet-saturated land). Numbers of 10,000 m^2 squares were positioned in the zones, the number employed based on obtaining a representative sample

TABLE 2. The distribution of Hot Spots (*HS*) among the 17 10,000 m^2 squares (identified as A–I) of the five zones of the two administrative villages. The data in brackets are the number of seasons that infected snails were found of the total number of seasons snails were collected, or could be collected, from the square.

Village	Zone	Structure	Square (hot spots)
Hexi	I Shamo	low-flat marshland	A B - *HS* (7 of 9) J
	II Houshan	higher-varied terrain	C D E - *HS* (6 of 9) F - *HS* (5 of 9) G - *HS* (5 of 9) H - *HS* (5 of 7) I - *HS* (6 of 6)
Jishan	I Village	once agricultural land	A - *HS* (7 of 9) D G - *HS* (8 of 8)
	II Southern	low-flat marshland	B C
	III Western	wet-saturated marshland	E - *HS* (5 of 9) F

from each zone. Each square was divided into 100 numbered cells, each 100 m^2. Each season, a random selection of 20 cells was made for each Square. All snails were collected from a 4 m^2 frame placed over the center of the selected cell. The position of each Square and selected cell was recorded each season using the GPS. All snails from a cell were crushed and the number of living snails and number of infected snails scored.

Hot spots (*HS* in Table 2) are those sites where infected snails were found in the majority of seasons, that is, two of three seasons or four of six seasons, and so on. We constantly examined the snail habitat areas for potential sites for hot spots. A special collection of several thousand snails was made from a small area where a hot spot was suspected and the snails crushed to determine if that spot might qualify for a routine collection.

As these snails are notorious for having a negative binomial distribution (i.e., they are highly clumped), we used SAS-8 to perform ANOVAS under a negative binomial model for snail counts. We examined trends through time and looked for significant differences between villages, zones and squares in snail density, infected snail density, and prevalence of infected snails. An offset of log (snail counts) was incorporated into ANOVA models for prevalence of infected snails. In the tables of trend analyses, all data are integrated across seasons. For example, seasons 2–9 (Table 4) does not mean comparing only season 2 data with data from season 9; 2–9 means including data from all seasons from 2–9. Significance is defined as $P \leq 0.05$. In the tables, S = significant difference, NS = not significant, and VS = very (highly) significant ($P \leq 0.01$). Deviation bars in all figures represent 95% confidence limits. Significant difference requires overlap not exceeding 30%, approximately. In some instances when numbers were small, the iterative procedure for fitting the negative binomial model did not converge. These are indicated in results tables by a “no fit” notation.

Mean values discussed in the text are arithmetic means based on the data taken from each cell of a collection. The data plotted in the figures are geometric means, that is, measures of central tendency as we expected changes in the data to occur in a relative fashion. The geometric mean estimates (and their confidence intervals) are obtained using the negative binomial modeling, which takes account of the spatial aggregation. The geometric means are useful summaries for highly skewed data such as found in this study. Thus, there will, in instances, be some small differences in mean values discussed in the text and those found in the figures.

RESULTS

Data from the two villages are compared: (1) for the entire village data, (2) among zones of each village, and (3) among hot spots in each village. The data types for each of the three area sets are snail density, density of infected snails, and prevalence of infected snails. A summary (Appendix 1) provides the snail density data and snail infectivity data in snails per m^2. A summary of all data for each of the five zones of the study is given in Appendix 2. Individual tables and figures involving the data are presented to make graphic presentation more easily displayed. As such, snail density is given as snails per frame (4 m^2), density of infected snails as number per 100 m^2 and prevalence of infected snails as number per 1,000 snails.

Snail Habitat without Snails

Data are presented primarily for the entire area of potential snail habitat. However, there are significant amounts of potential snail habitat that have no snails. There are significant differences among zones in no-snail areas (Fig. 1). Jishan Southern and Hexi Shamo are the most similar in that during both the first half and second half of the seasons studies, less than 4% of the area had no snails. Jishan Village was the most volatile zone with nearly 40% of the area having no snails in the first five seasons, dropping to 21% in the last four seasons. Jishan Western Zone and Hexi Houshan Zone were rather similar, with 25 to 32 % area having no snails in seasons 1–5, dropping to about 5% in seasons 6–9.

In examining the critical hot spots in each village, we also calculate and present the numbers for areas that only had snails given that areas with snails were so patchy in some zones. Data for each hot spot square are plotted as numbers for only those frames that had snails in Figs. 17–22.

Comparing Villages

Snail Density: Changes in the mean number of snails per frame for each season in both villages are shown in Fig. 2. Aside from seasons 2, 3, and 7, the average numbers per

TABLE 3. Trends from seasons 1 to 9 in density of snails per frame (4 m^2) in the two villages: Statistics and probabilities.

	Jishan						Hexi					
Season	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9		4.75	2.3	7.13	0.0002	VS		4.75	2.3	7.13	0.0002	VS
2 - 9	6.45		2.31	10.56	0.002	VS		1.27	-1.74	4.2	NS	
3 - 9	4.87		0.27	9.67	0.0376	S		6.09	3.21	8.88	<0.0001	VS
4 - 9	5.53		-0.27	11.66	0.062	NS		2.07	-1.5	5.53	NS	
5 - 9		0.7602	-6.144	7.22	NS			4.77	0.53	8.82	0.0279	S
6 - 9	3.907		-4.966	13.61	NS		9.37		3.3	15.8	0.0021	VS
7 - 9	84.82		59.37	114.32	<0.0001	VS	121.69		102.2	143.1	<0.0001	VS
8 - 9	172.68		100.033	271.72	<0.0001	VS	173.68		128.8	227.3	<0.0001	VS

TABLE 4. Comparison of villages for density of infected snails over nine seasons.

	Jishan						Hexi					
Season	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9		14.87	6.6	22.4	0.0007	VS	9.28		-1.25	20.93	0.0859	NS
2 - 9		20.96	12.82	28.35	<0.0001	VS	12.85		-0.28	27.72	0.05577	NS
3 - 9	0.145		-12.88	15.12	NS		13.63		-1.78	31.46	0.08570	NS
4 - 9		3.3	-15.51	19.05	NS		5.1		-10.77	23.8	NS	
5 - 9		20.39	2.08	35.28	0.0308	S		7.4	-12.84	24.01	NS	
6 - 9		11.83	-18.98	34.66	NS			16.07	-7.83	34.66	NS	
7 - 9	198.6		48.07	502.16	0.0022	VS	54.6		-1.54	142.7	0.05841	NS
8 - 9	242.86		6.98	998.82	0.0381	S		14.29	-89.74	61.28	NS	

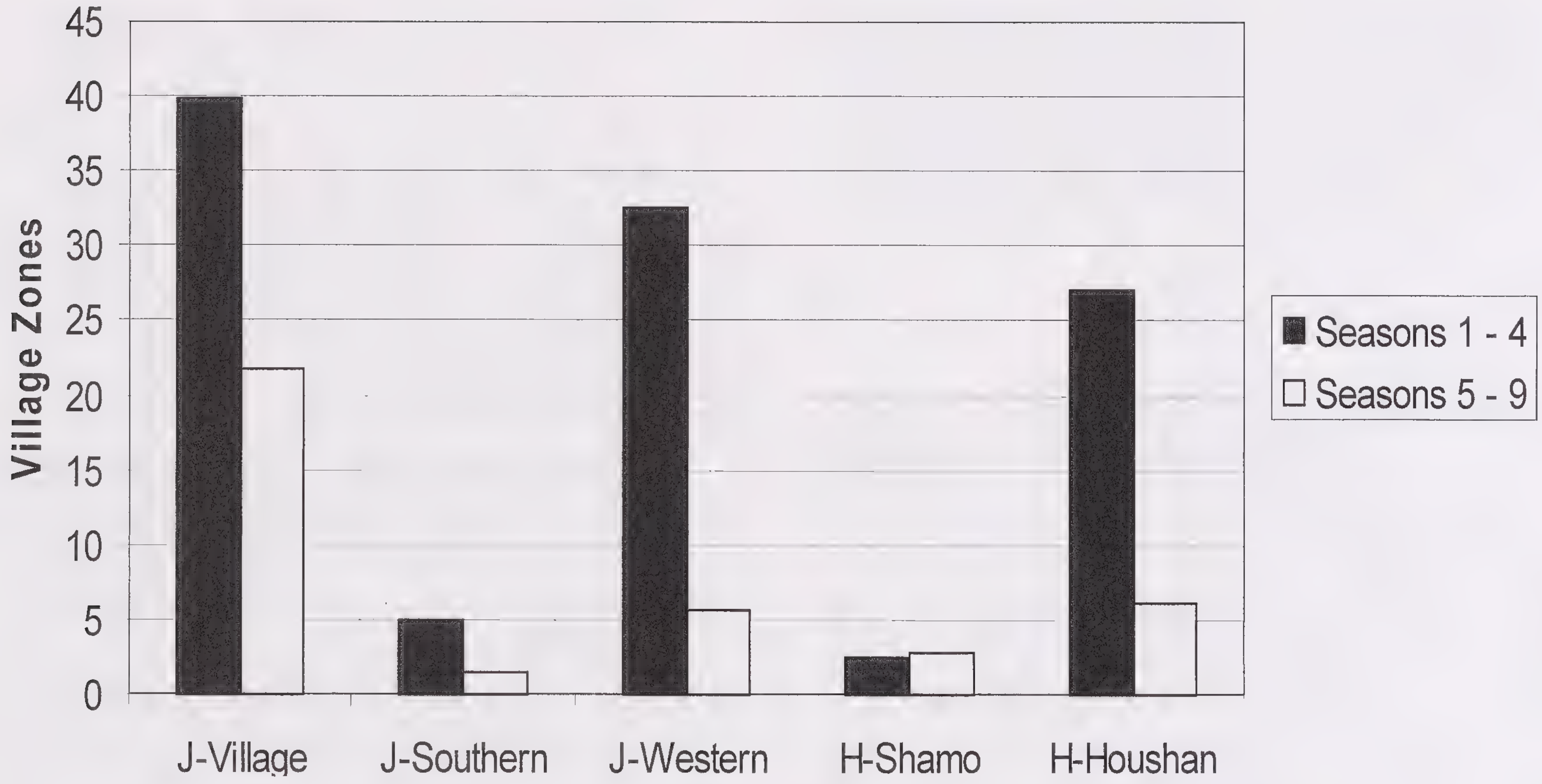

FIG. 1. Percentage of area without snails seasons 1–4 and seasons 5–9.

area each season did not differ significantly between villages. Over the nine seasons, the mean number ± standard deviation of snails per m^2 in Jishan was 10.2 ± 5.3. In Hexi, it was 9.8 ± 5.1 (mean of means for each season). Trends through time are assessed in Table 3. Numbers per m^2 ranged from 4.42–20.00 in Jishan, with the greatest number in season 1. In Hexi, the range was 2.72–15.74 with the greatest number in season 1 (Appendix 1).

Density of Infected Snails: At study end there were no significant differences between villages in densities of infections (comparing seasons 1 and 9) (Fig. 3). Significant points are: (1) There was a big spike of infections in Jishan (intervention village) in season 2 not paralleled in Hexi. (2) Infection levels dropped in seasons 3 and 4 to levels not significantly different from those seen in the baseline data (season 1). (3) There were spikes of infection

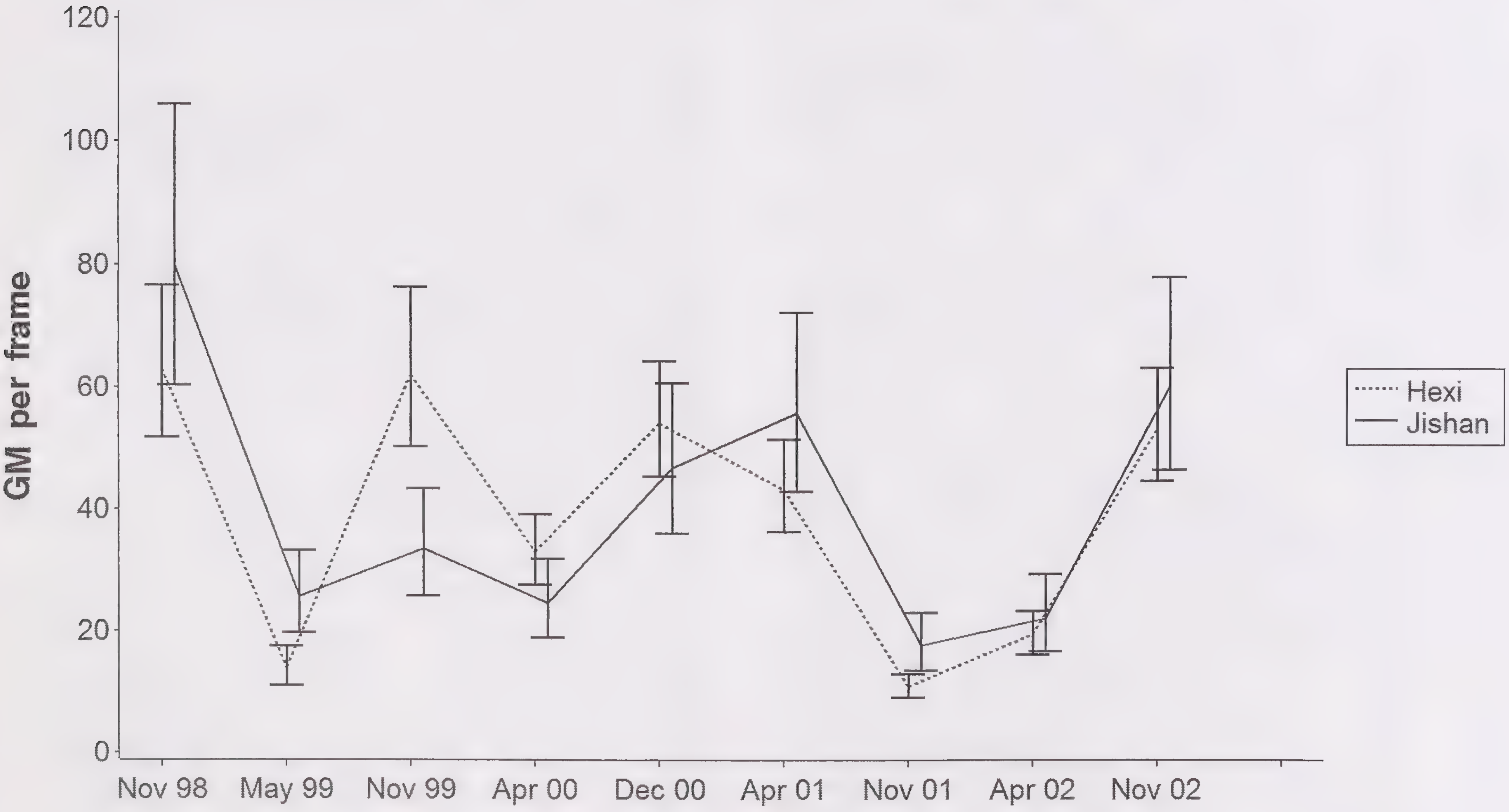

FIG. 2. Comparison of villages for density of snails employing the geometric mean per frame for nine seasons.

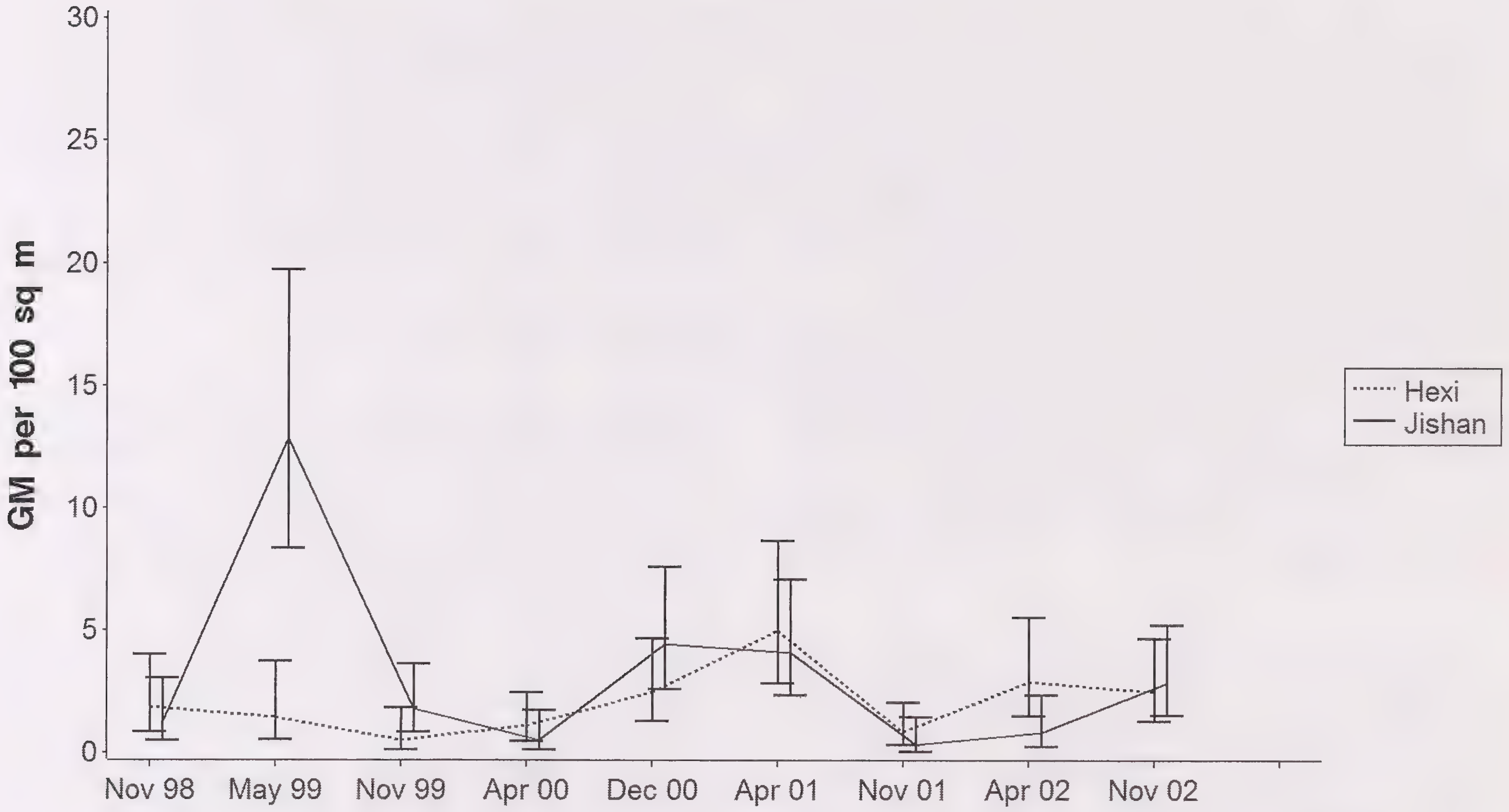

FIG. 3. Comparison of villages for density of infected snails employing the geometric mean per 100 m^2 for nine seasons.

in seasons 5 and 6 (Dec. 2000; Apr. 2001). (4) There were significant drops in infections in season 7 in both villages. (5) As seen in Table 4, there were significant decreases in density of infected snails in Jishan not seen in Hexi comparing seasons 1 to 9 (15%; very significant, P = 0.0007), 2 to 9 (VS), and 5 to 9. However, these are due to the unique and pronounced spike of infections in Jishan in seasons 2 and 5, not to the effects of intervention.

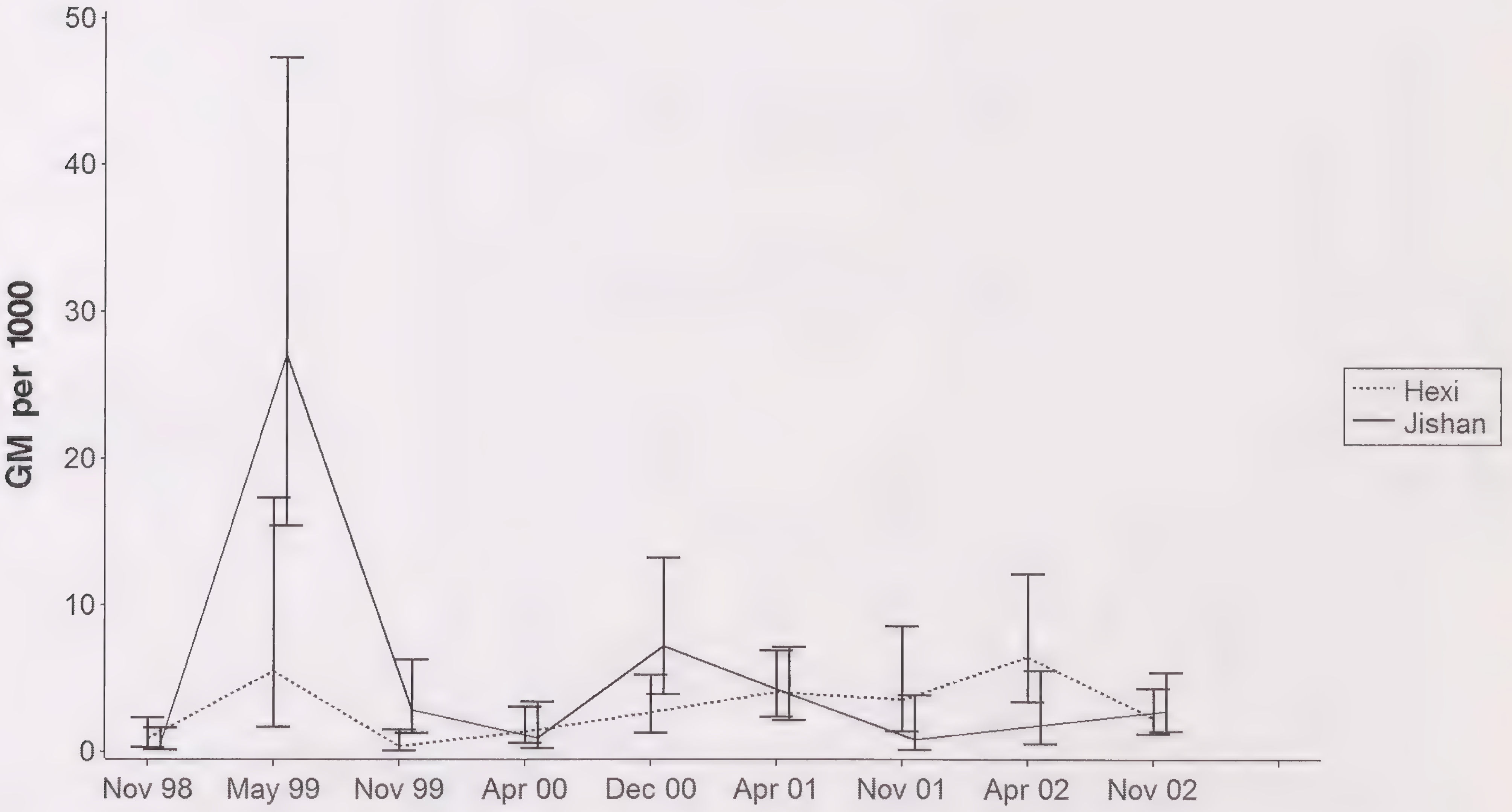

FIG. 4. Comparison of villages for prevalence of infected snails employing the geometric mean per 1,000 snails for nine seasons.

Additionally, there was a very significant increase of infection in Jishan from season 7 to 9 (VS) and 8 to 9 (S). Of importance was the occurrence of a severe drought during season 7 and that density of snails and density of infected snails alike dropped considerably in both villages due to inability to collects snails buried in the soil to escape the drought.

In the control village (Hexi), there was no sharp spike of infection in season 2; there was an increase of density of infection in seasons 5 and 6 paralleling that seen in Jishan. There were no significant differences between villages in density of infected snails any season except season 2.

Prevalence of Infected Snails: Villages are compared in prevalence of infected snails (Fig. 4, Table 5). Except for seasons 2 and 3, there were no significant differences. In Jishan, prevalence was lowest in season 1 (0.6/1,000 snails) and highest in season 2 (20/1,000 snails). In Hexi, the lowest was in season 3 (0.3/1,000 snails) and highest in season 8 (6.0/1,000 snails).

In summary, the more important data are the density of infected snails. How many infected snails are found per unit area? Excluding seasons 2 and 7 for Jishan for reasons given above, the greatest was 4.5/100 m^2 in season 5 and least was 0.8/100 m^2 in season 8, but rebounding to 2.9/100 m^2 in the last season. In Hexi the greatest was 5.0/100m^2 in season 6 and least was 0.5/100 m^2 in season 3. Snail density in Jishan was greatest in season 1 (20/m^2) (excluding season 7) and least in season 9 to 5.5/m^2. In Hexi, the greatest was in season 1 (15.7/m^2) and least in season 2 (3.5/m^2).

Comparing Zones

Snail Density (Numbers per Frame): Results for the zones clearly show striking differences among zones in both villages (compare Figs. 5 and 6). In Jishan, zone II, the Southern Zone, had more than double the number of snails in the other two zones; in seasons 1, 5 and 9, more than three times. Numbers of snails in zones I and III were parallel through the seasons excepting the sharp drop in the Western Zone in season 2. As seen later, the sharp upswings in zone II snail density in seasons 1, 5, and 9 had no correspondence to the density of infected snails at those times. In Hexi, zone I (Shamo Grazing land) was similar to zone II (Southern Grazing land) of Jishan in having high density compared to zone II (Houshan). The gap, > 3 to 1 was reduced to no significant difference between zones in seasons 6–9. The average densities per zone in m^2 over all seasons were as follows. High density zones: Jishan-Southern x Hexi-Shamo:

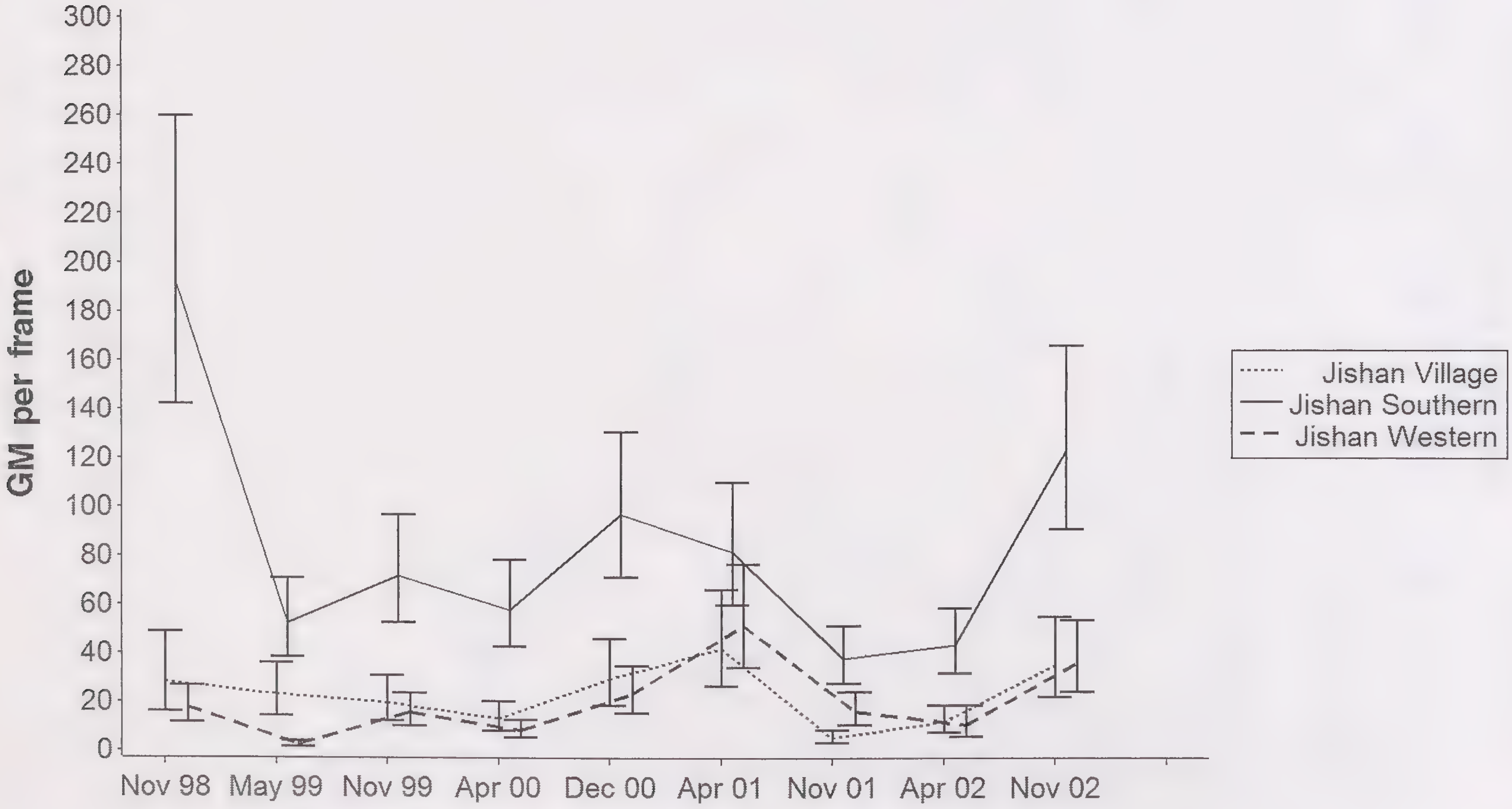

FIG. 5. Comparison of three zones in Jishan Village for density of snails employing the geometric mean per frame for nine seasons.

TABLE 5. Comparison of villages for trends of prevalence of infected snails over nine seasons.

	Jishan						Hexi					
Season	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9		15.09	5.03	24.1	0.00421	VS	17.32		4.77	31.36	0.00566	VS
2 - 9		23.22	13.91	31.52	<0.0001	VS	13.09		-1.48	29.81	0.08051	NS
3 - 9		5.31	-13.82	21.23	NS		22.08		4.39	42.76	0.01248	S
4 - 9		9.08	-14.58	27.85	NS		11.85		-5.66	32.62	NS	
5 - 9		22.18	-0.55	39.78	0.05508	NS	0.16		-18.7	23.37	NS	
6 - 9		7.896	-33.26	36.34	NS			11	-14.0	30.53	NS	
7 - 9	79.1		-23.58	319.74	NS			26.64	-20.7	55.42	NS	
8 - 9	55.55		-65.86	608.82	NS			63.66	13.62	84.71	0.02195	S

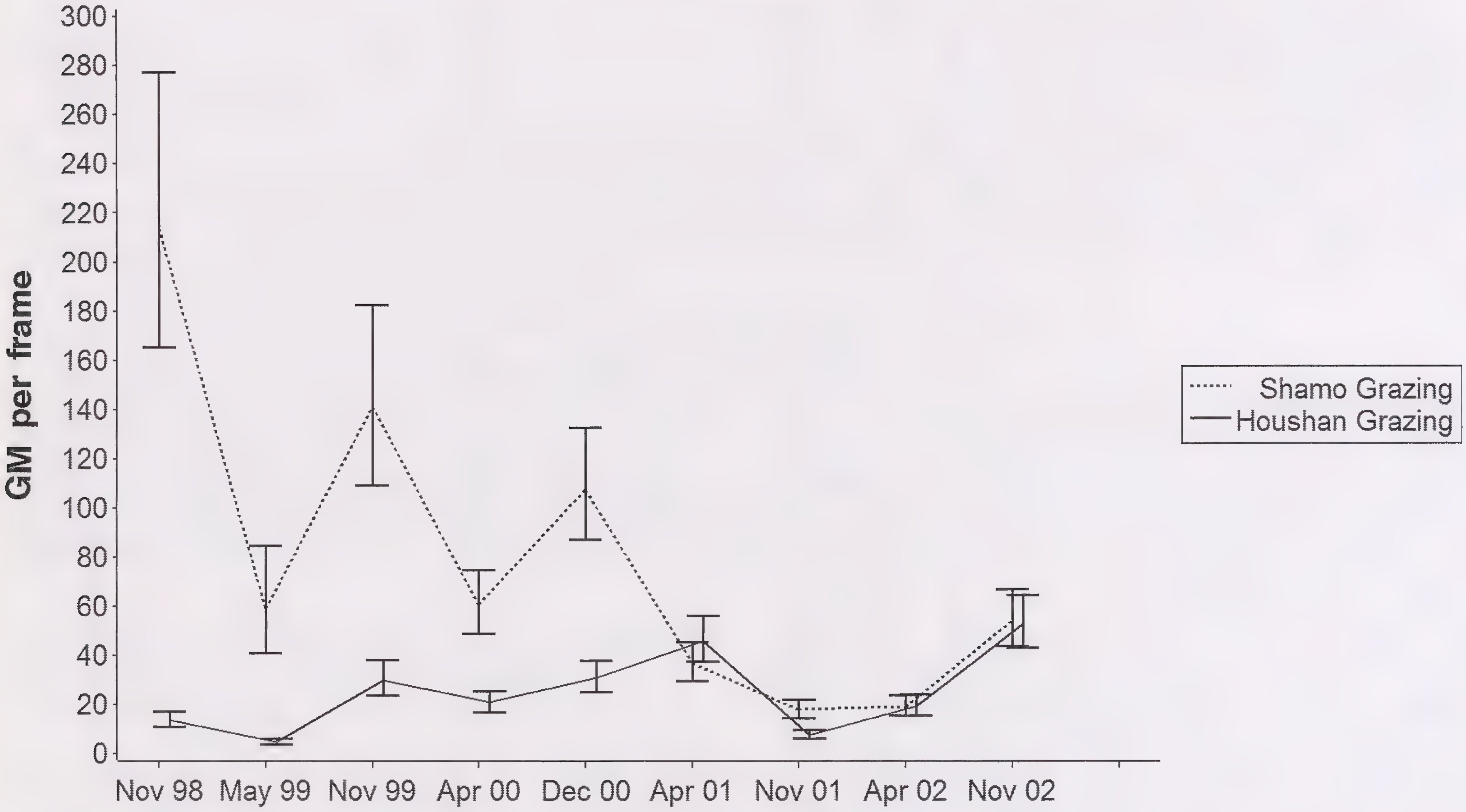

FIG. 6. Comparison of two zones in Hexi Village for density of snails employing the geometric mean per frame for nine seasons.

20.97 ± 12.20; 19.75 ± 16.15. Low density zones: Jishan Village and Western Zones = 5.77 ± 2.94; 4.51 ± 3.44; Hexi Houshan Zone = 6.31 ± 4.08.

Density of Infected Snails (Numbers per 100 m²): In Jishan (Fig. 7), a sharp jump in density in season 2 was most pronounced in the Village and Southern Zones, with densities

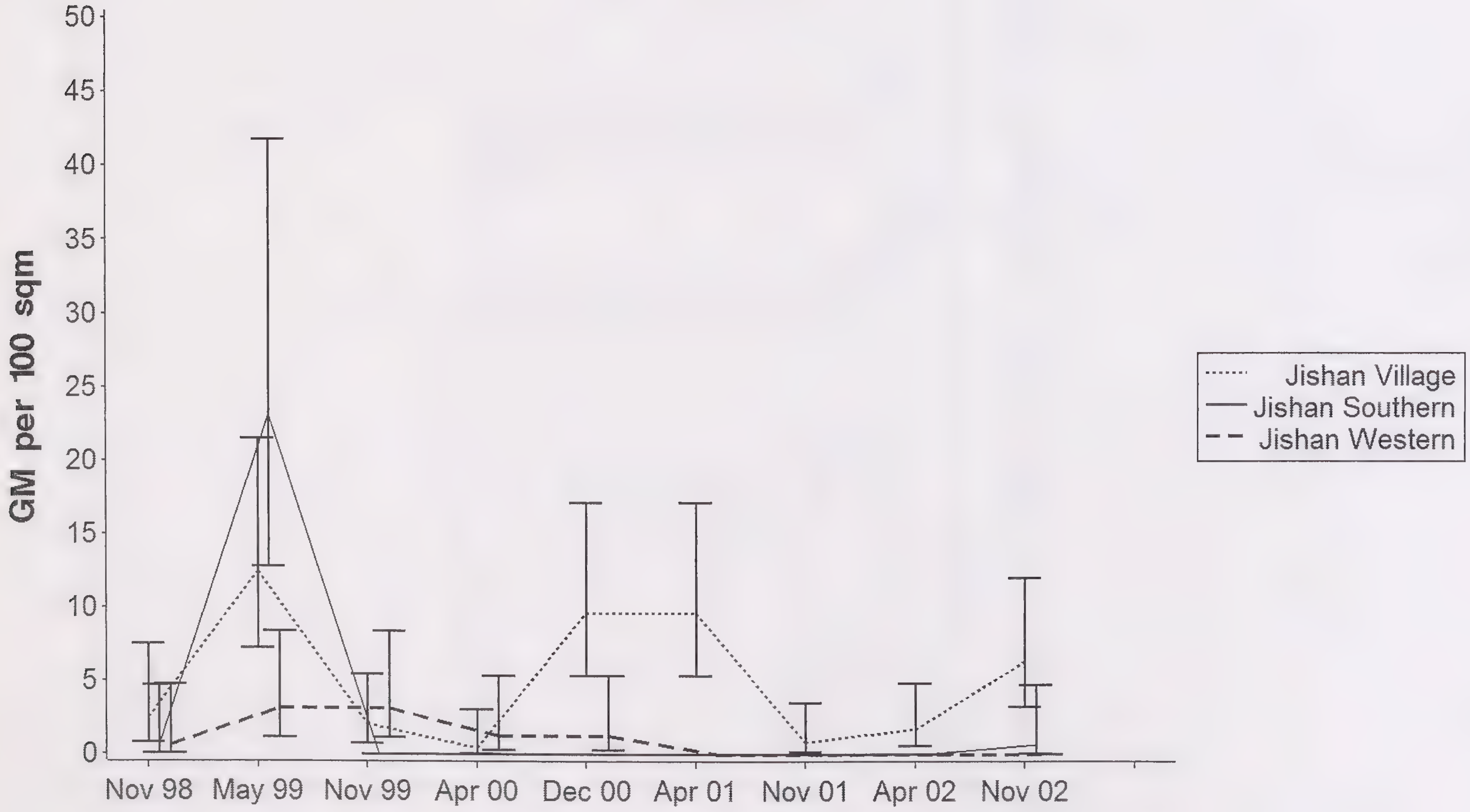

FIG. 7. Comparison of three zones in Jishan Village for density of infected snails employing the geometric mean per 100 m² for nine seasons.

TABLE 6. Zones of villages compared for trends in density of infected snails over nine seasons.

	Jishan							Hexi						
Season	Zone	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	Zone	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9	Village		5.04	-5.75	14.73	NS		Shamo		29.21	9.046	44.90	0.0069	S
	Southern		55.56	31.18	71.31	0.0003	VS	Houshan	23.41		9.7805	38.74	0.0004	VS
	Western		32.39	7.92	50.36	0.0130	S							
2 - 9	Village		8.96	-1.96	18.72	NS		Shamo		13.095	-31.74	42.67	NS	
	Southern		77.72	53.89	89.24	<0.0001	VS	Houshan	16.52		2.46	32.50	0.0198	S
	Western		47.76	20.56	65.65	0.0024	S							
3 - 9	Village	5.78		-9.00	22.96	NS		Shamo		20.174	-24.50	48.82	NS	
	Southern	NI						Houshan	18.32		1.37	38.10	0.0329	S
	Western		59.39	20.18	79.34	0.0090	S							
4 - 9	Village		2.13	-17.65	18.58	NS		Shamo		30.00	-22.71	60.07	NS	
	Southern	NI						Houshan	9.12		-8.10	29.56	NS	
	Western	no fit												
5 - 9	Village		21.76	3.64	36.47	0.0209	S	Shamo		50.52	-3.47	76.34	0.0616	NS
	Southern	NI						Houshan		2.54	-19.92	20.79	NS	
	Western	no fit												
6 - 9	Village		15.67	-13.81	37.52	NS		Shamo		63.46	-26.7	89.46	NS	
	Southern	NI						Houshan		12.34	-13.23	32.13	NS	
	Western	NI												
7 - 9	Village	no fit						Shamo		no fit				
	Southern	NI						Houshan	62.93		2.7	158.50	0.0382	S
	Western	NI												
8 - 9	Village	no fit						Shamo	NI					
	Southern							Houshan		14.29	-90.54	61.44	NS	
	Western													

reaching 12.5/100 m^2 (0.125/m^2) and 23.1/100 m^2 (0.231/m^2) respectively. Thereafter, densities declined dramatically in season 3 in the Southern Zone and were zero until the last season when infections re-emerged. Densities decline in the Western Zone from season 3 to disappear in season 6, thereafter not to reappear. By contrast, there were no significant changes in density in the Village Zone comparing seasons 1, 3, 4, but a significant increase from season 4 to seasons 5 and 6, jumping from 0.425/100 m^2 to 9.57/100 m^2. The levels in seasons 7 and 8 were not significantly different from those seen in seasons 1, 3, and 4. In season 9 there was a rise to a mean of 6.25/100m^2 (contrasted with 2.5/100 m^2 in season 1).

Considering significant changes in the Village Zone (Table 6), the only significant trend following the spike in season 2, was from season 5 to 9 (P = 0.021) following the elevated spike of infection in season 5 and maintained in season 6. Considering the other two zones, from season 3 onward, there were obviously significant decreases in the other two zones as numbers of infected snails went to zero in season three in the Southern Zone; in season 6 in the Western Zone.

Densities of infected snails in Hexi Village (two zones) are shown in Fig. 8. With the exceptions of seasons 1, 2, and 9, there were no significant differences between the zones. However, the average densities were generally higher in the Houshan Zone. The mean of means over nine seasons in the Shamo Zone was 1.37 ± 2.22 infected snails per 100 m^2) contrasted with 2.44 ± 2.02 in the Houshan Zone. Both the Hexi Zones are lower than the 3.79 ± 3.68 found in the Jishan Village Zone.

There was no spike in numbers of infected snails in the second season in Hexi. There was a significant decline in density of infected snails in Shamo from season one to nine (Table 6). Thereafter, there were no significant changes in density until the last season when numbers of infected snails dropped to zero. In the Houshan grazing range, there was a contrasting result. There were significant increases in density of infected snails comparing seasons, 1, 2 and 3 to season 9; and season 7 to 9.

Prevalence of Infected Snails: Prevalence's do not precisely mimic trends of density (contrast Figs. 7 and 9 for Jishan Village). As with density data, focus is on the Village Zone. Isolating the effect of the spike in season 2, there was no significant difference among seasons except for that involving season 5 in which there was a pronounced increase in prevalence from season 4. The only significant trend decrease in prevalence was from season 5 to season 9 (21.37%; P = 0.03; Table 7).

In Hexi, there was a spike in prevalence of infected snails in season 2 in the Houshan Zone (Fig. 10). While it would appear that there was a slow increase in prevalence in the Houshan

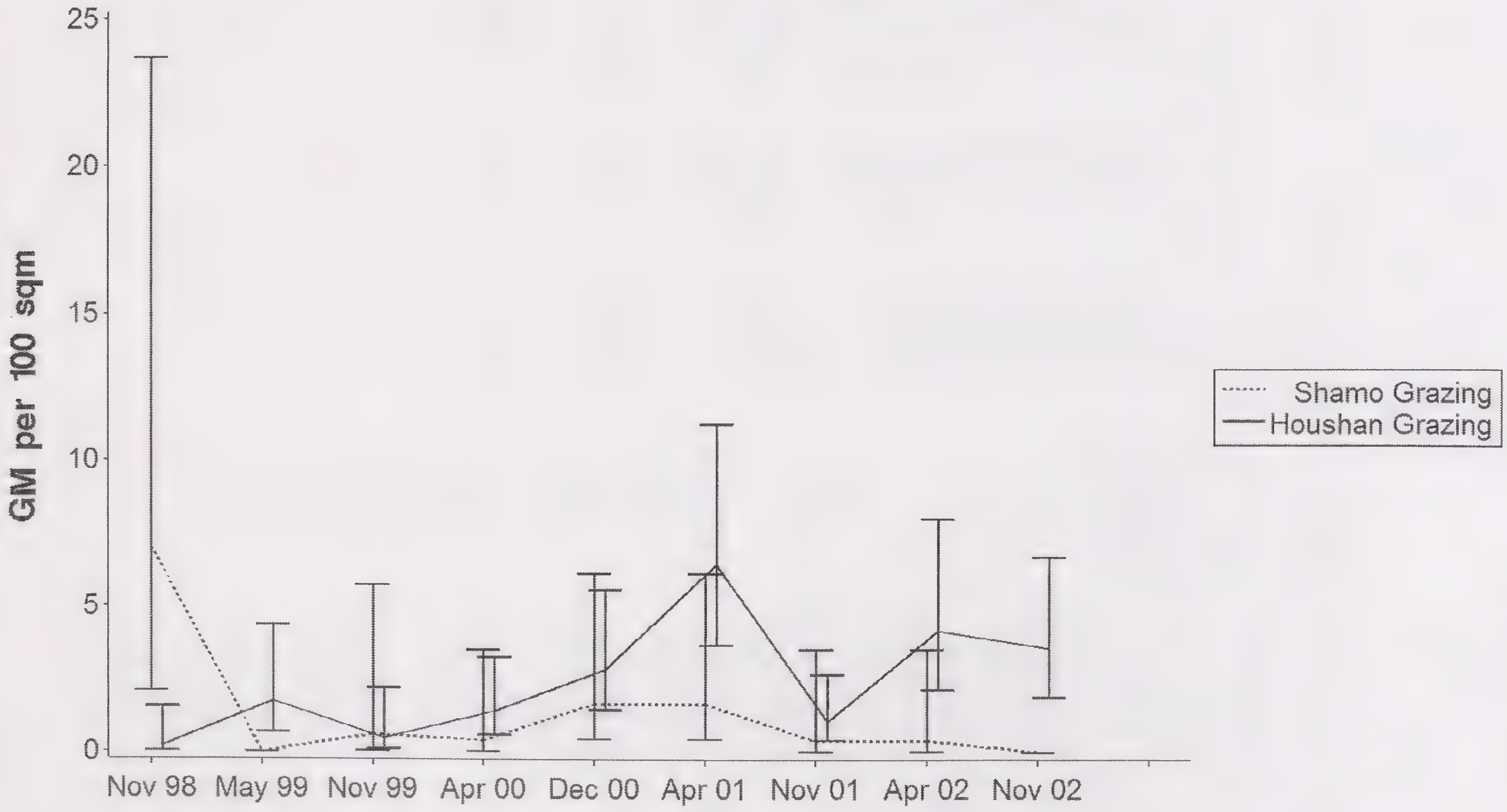

FIG. 8. Comparison of two zones in Hexi Village for density of infected snails employing the geometric mean per 100 m^2 for nine seasons.

TABLE 7. Comparing zones of the villages for trends in prevalence of infected snails over nine seasons.

	Jishan							Hexi						
Season	Zone	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P	Zone	% Inc/ Season	% Dec/ Season	Lower Limit	Upper Limit	P Value	P
1 - 9	Village		4.66	-6.82	14.91	NS		Shamo		5.07	-19.47	24.566	NS	
	Southern		56.51	31.00	72.59	0.0004	VS	Houshan	12.35		-0.83	27.274	0.067	NS
	Western		54.20	26.42	71.49	0.0013	VS							
2 - 9	Village		10.09	-1.25	20.16	0.0793	NS	Shamo	15.28		-22.87	72.291	NS	
	Southern		84.38	66.34	92.75	<0.0001	VS	Houshan	6.33		-7.268	21.931	NS	
	Western		70.03	50.40	81.89	<0.0001								
3 - 9	Village	2.14		-13.37	20.41	NS		Shamo	15.28		-22.87	72.291	NS	
	Southern	no fit						Houshan	6.33		-7.268	21.931	NS	
	Western		67.86	36.01	83.86	0.0012	S							
4 - 9	Village		4.90	-15.49	21.69	NS		Shamo	9.97		-27.47	66.715	NS	
	Southern	no fit						Houshan	15.48		-1.505	35.248	0.076	NS
	Western		88.89	24.72	98.36	0.02	S							
5 - 9	Village		21.37	1.91	36.96	0.03	S	Shamo		4.62	-58.19	42.488	NS	
	Southern		no fit					Houshan	7.23		-9.794	27.47	NS	
	Western			99.96	-4.87	100.00	NS							
6 - 9	Village			16.73	-13.24	38.77	NS	Shamo		22.39	-46.73	58.945	NS	
	Southern		no fit					Houshan		1.51	-21.65	20.266	NS	
	Western		no fit											
7 - 9	Village		18.10		-45.57	156.23	NS	Shamo		74.58	-116.1	97.01	NS	
	Southern		no fit					Houshan		29.94	-15.91	57.655	NS	
	Western													
8 - 9	Village		35.07		-62.91	391.91	NS	Shamo	no fit					
	Southern		no fit					Houshan		62.32	10.405	84.154	0.0272	S

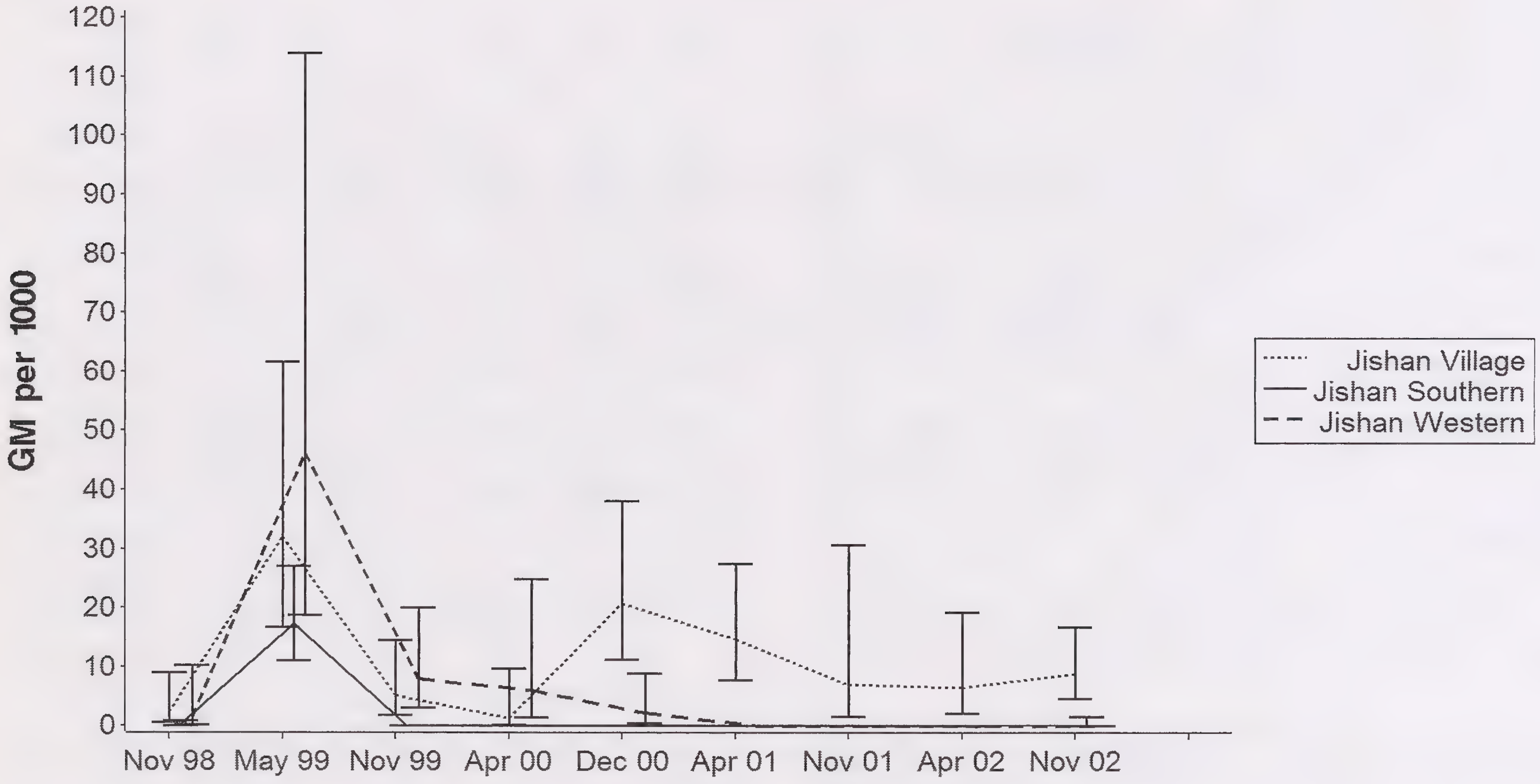

FIG. 9. Comparison of three zones in Jishan Village for prevalence of infected snails employing the geometric mean per 1,000 snails for nine seasons.

Zone from season 3 to 9, there was no significant trend, except for a significant decrease from season 8 to 9 (Table 7, 62%; P = 0.027).

Hot Spots

The persistence of infected snails within a 10,000 m^2 area has profound implications for the epidemiology of schistosomiasis (Table 2). In Jishan, two of three such areas were hot spots in the Village Zone closest to the main cluster of human habitation and frequently visited by villagers and buffaloes. The Western Zone, far removed from the natural villages, had one hot spot that existed only 5 of 9 seasons (disappearing by the 6th season). By contrast, Hexi had more hot spots with 5 of 7 squares in the Houshan Zone and one of three in Shamo.

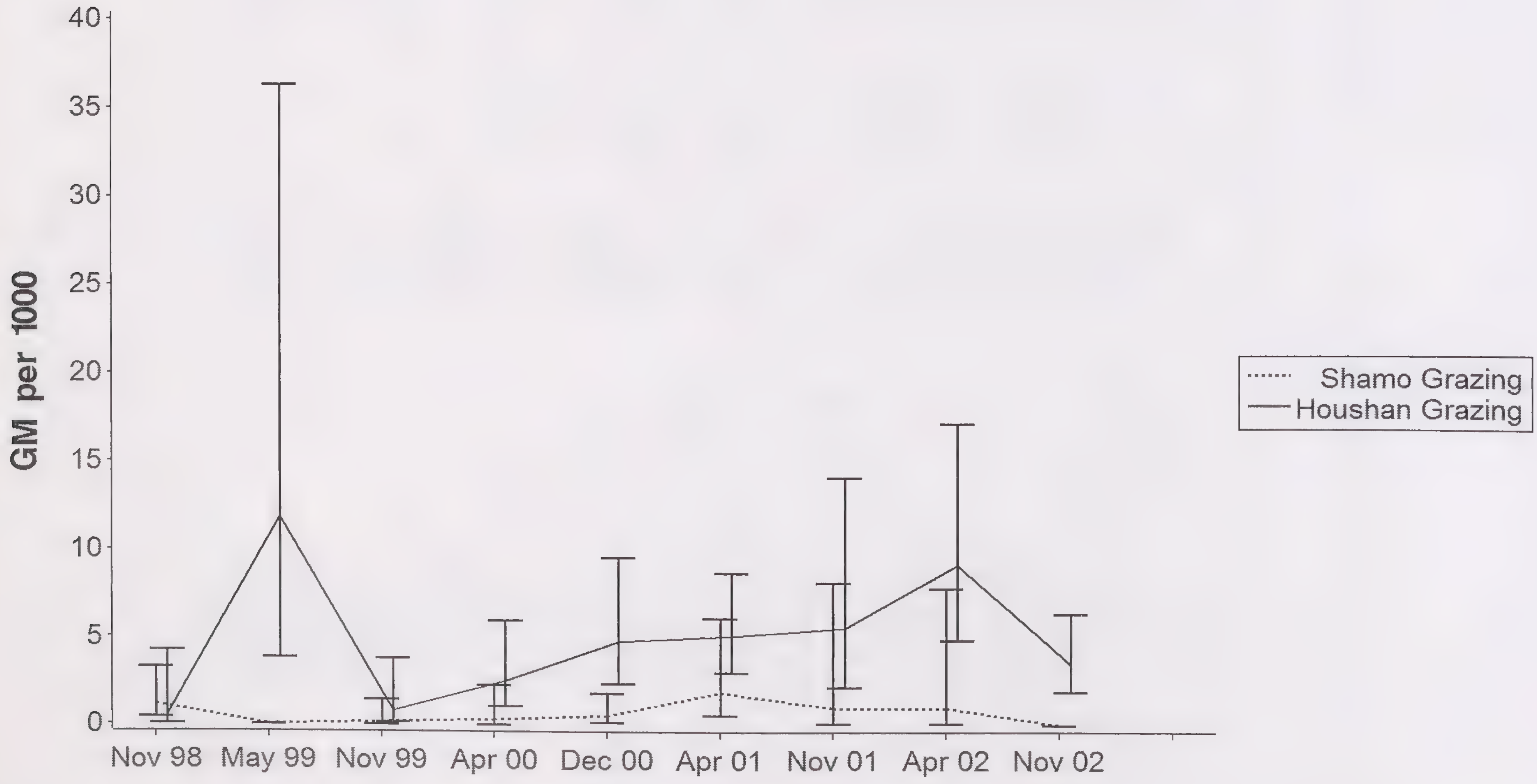

FIG. 10. Comparison of two zones in Hexi Village for prevalence of infected snails employing the geometric mean per 1,000 snails for nine seasons.

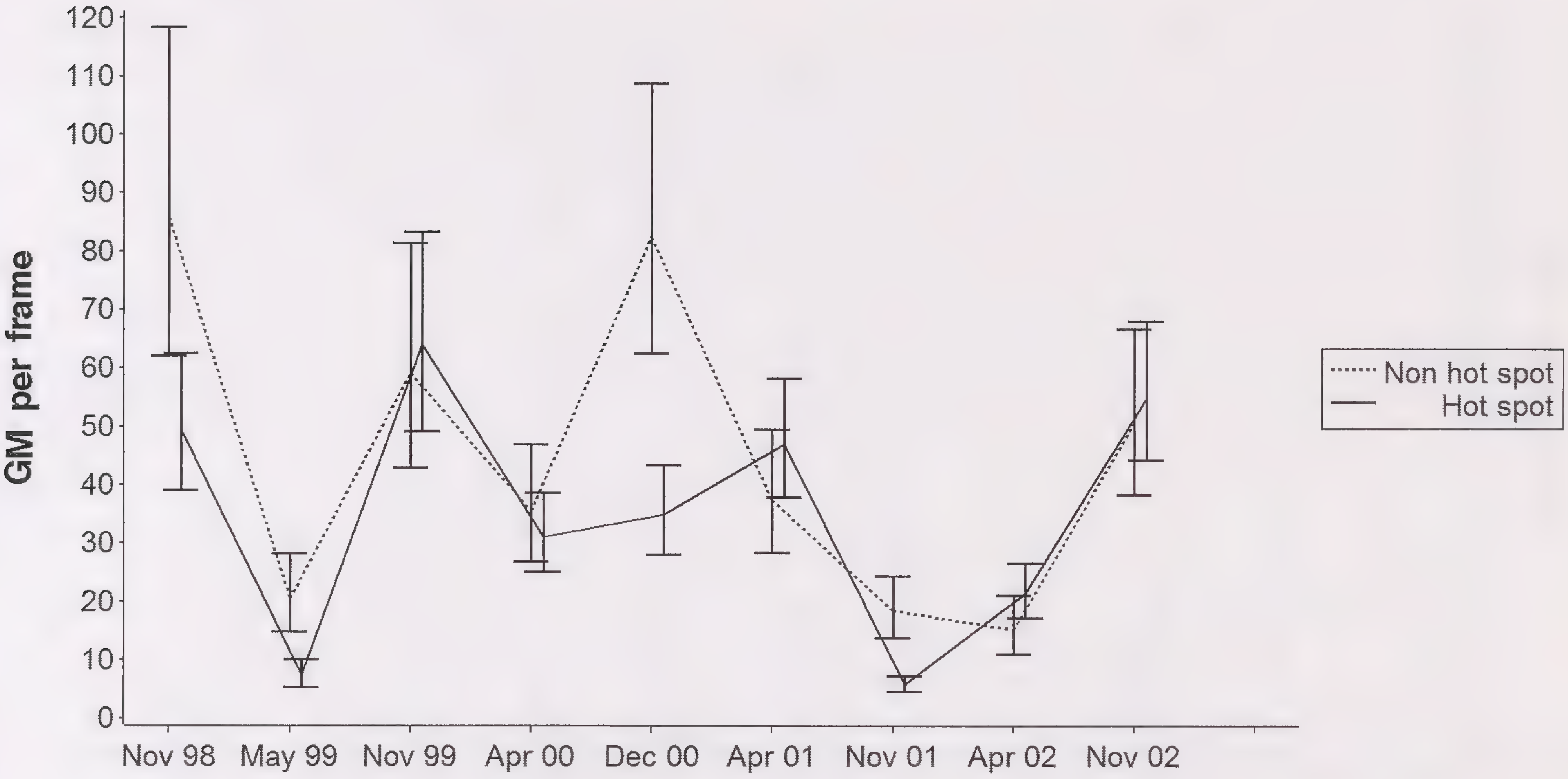

FIG. 11. Comparison of snail densities in Jishan Village for hot spots vs. non hot spots employing geometric mean per frame.

Hot Spots vs. Non Hot Spots Villages: The two villages differed in density of snail hot spots vs. non hot spots (Figs. 11, 12). In Jishan, there were significantly more snails per area in non hot spots than in hot spots (average of averages of snails per frame over the nine seasons of 49.3 ± 25.4/frame vs. 28.2 ± 16.6 per frame). Of note was the surge of snails per frame in seasons 5 and 6. In Hexi, there was no significant difference except in season 5. The average of averages of snails per frame over the nine seasons was 38.5 ± 21.9 in hot spots vs. 45.7 ± 33.7 in non hot spots.

Density of Infected Snails in Hot Spots vs. Non Hot Spots: Compared to density of snails, the situation of density of infected snails is quite different in that there were significantly more

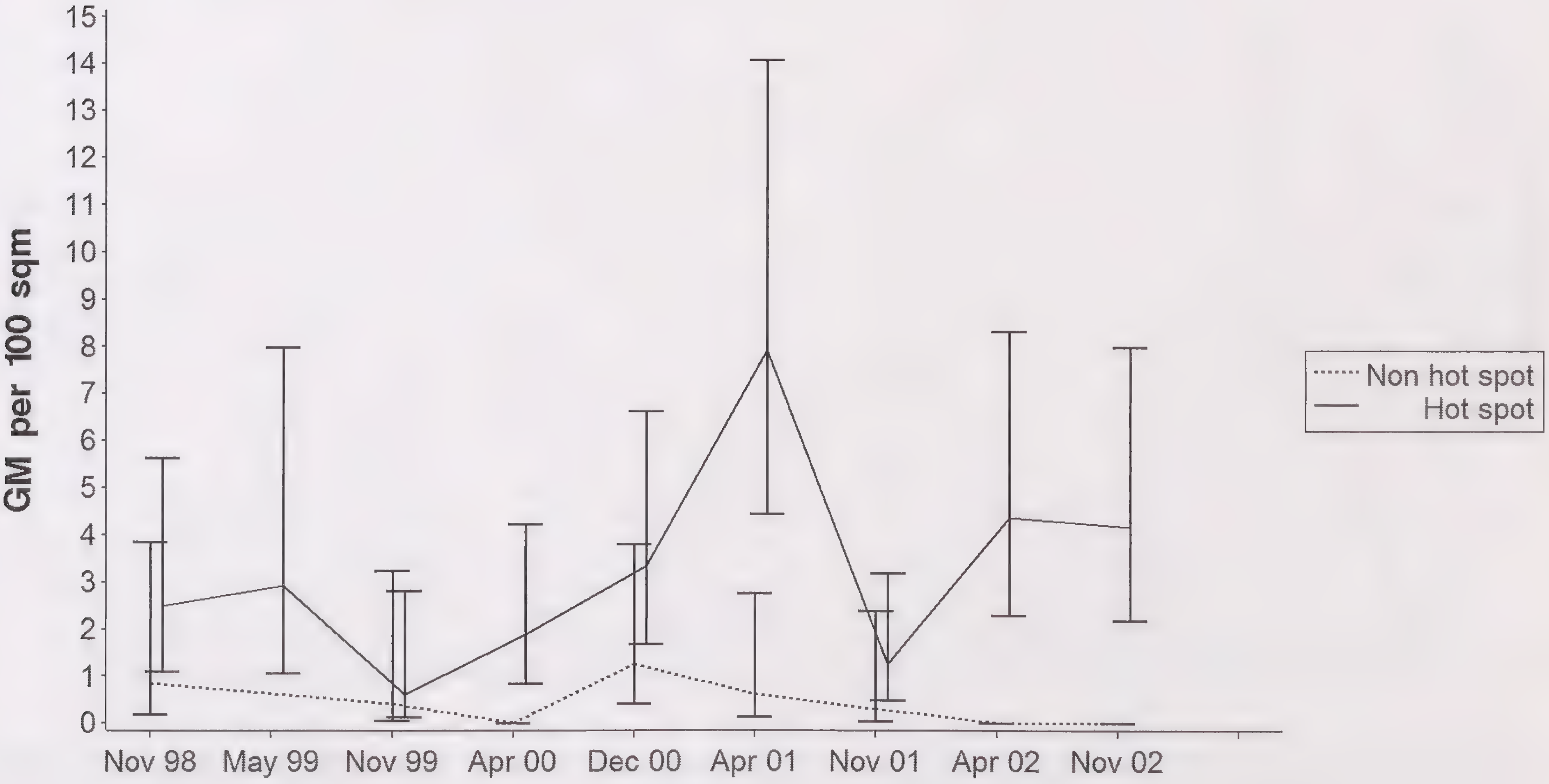

FIG. 12. Comparison of snail densities in Hexi Village for hot spots vs. non hot spots employing geometric mean per frame for nine seasons.

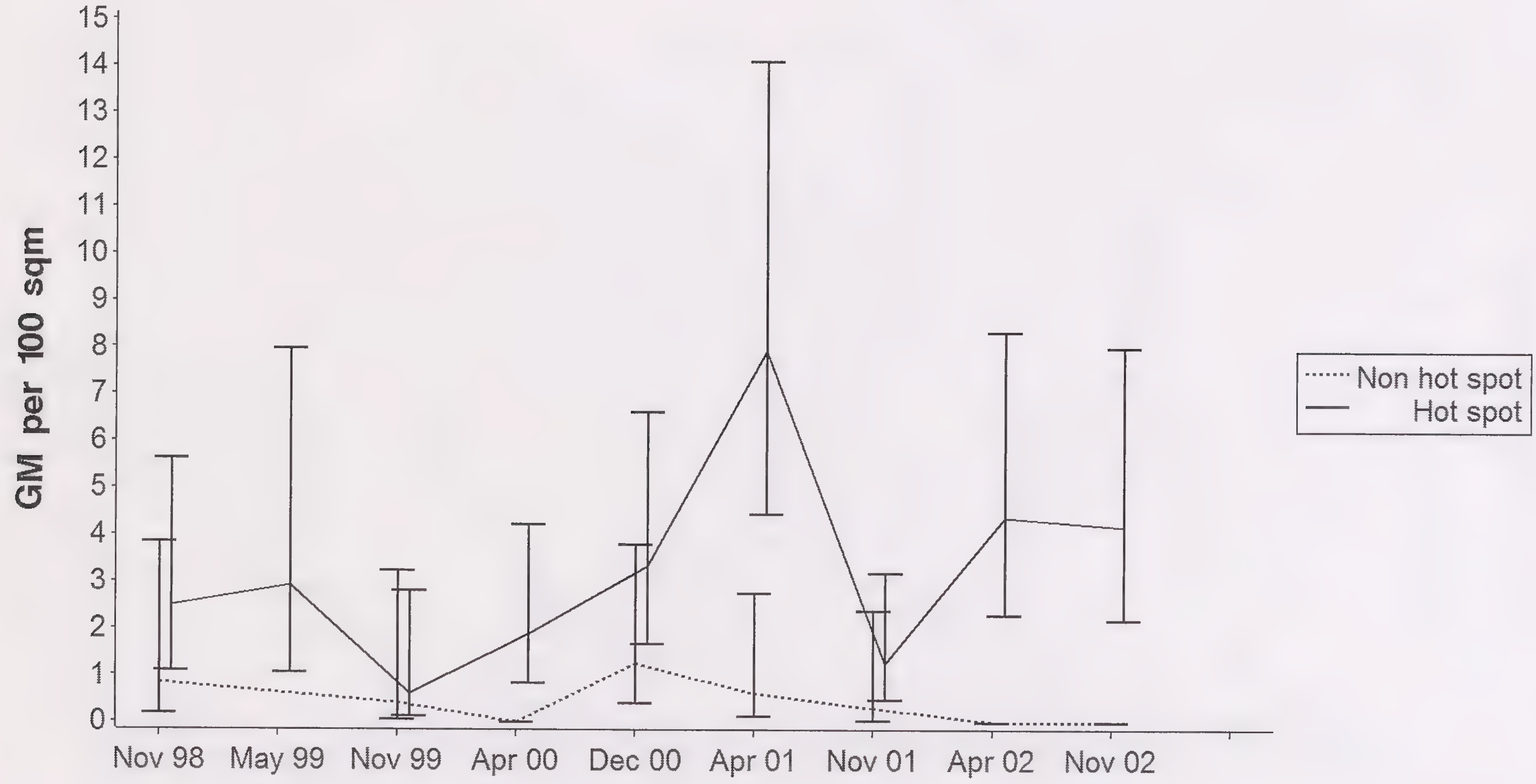

FIG. 13. Comparison of density of infected snails in Hexi Village for hot spots vs. non hot spots employing geometric mean per 100 m² for nine seasons.

infected snails per area in hot spots than in non hot spots. The mean of means over nine seasons was 3.5 infected snails per 100 m² ± 2.0 in hot spots vs. 0.84 ± 0.91 in non hot spots in Hexi (Fig. 13). For Jishan, the comparative data were 5.3 ± 4.7 in hot spots; 1.7 ± 3.7 in non hot spots. The high standard deviations reflect the sharp spike of infected snails in season 2 (Fig. 14).

Prevalence of Infected Snails in Hot Spots vs. Non Hot Spots: The difference in number of snails per 1,000 snails found in hot spots

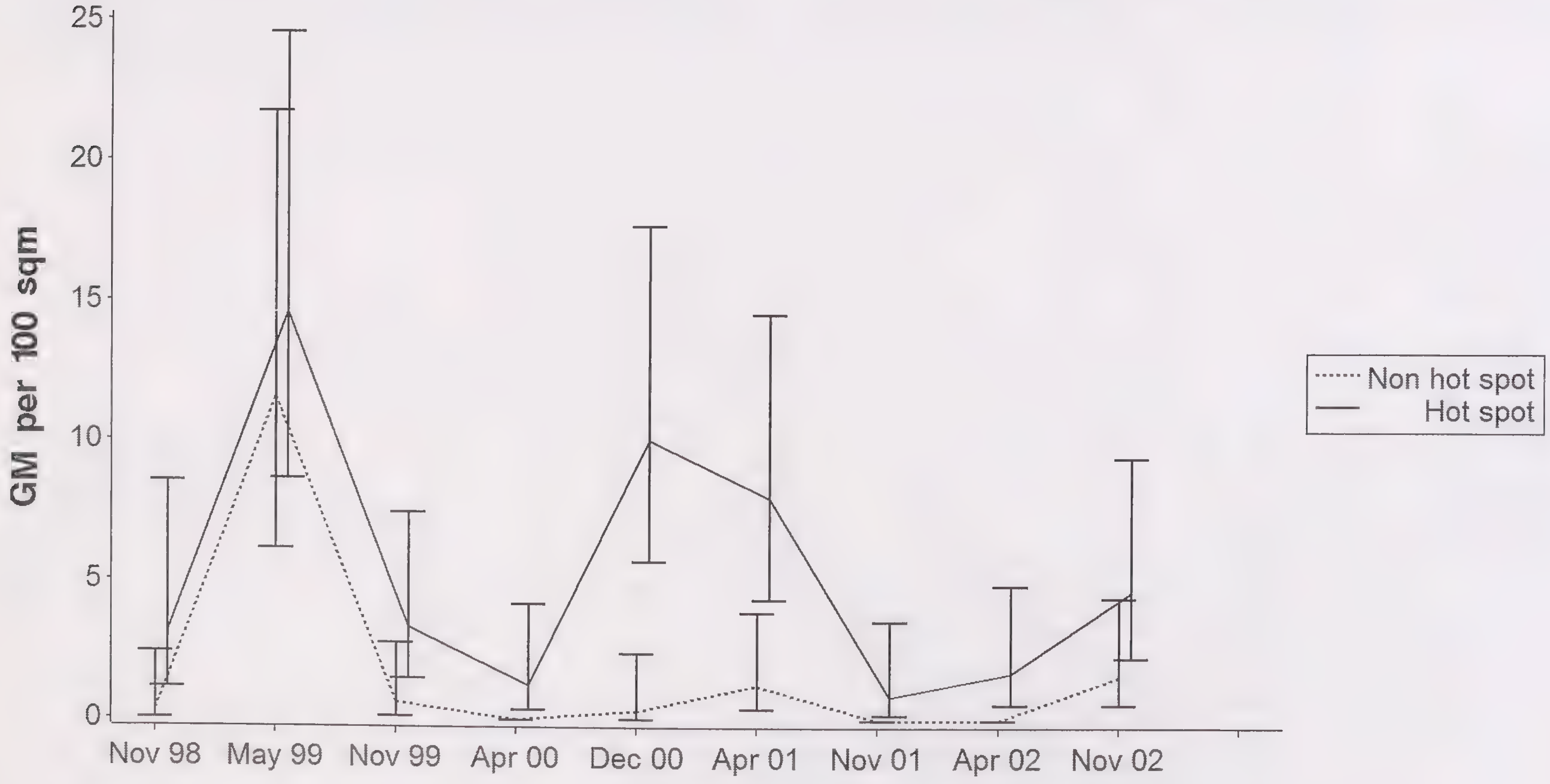

FIG. 14. Comparison of density of infected snails in Jishan Village for hot spots vs. non hot spots employing geometric mean per 100 m² for nine seasons.

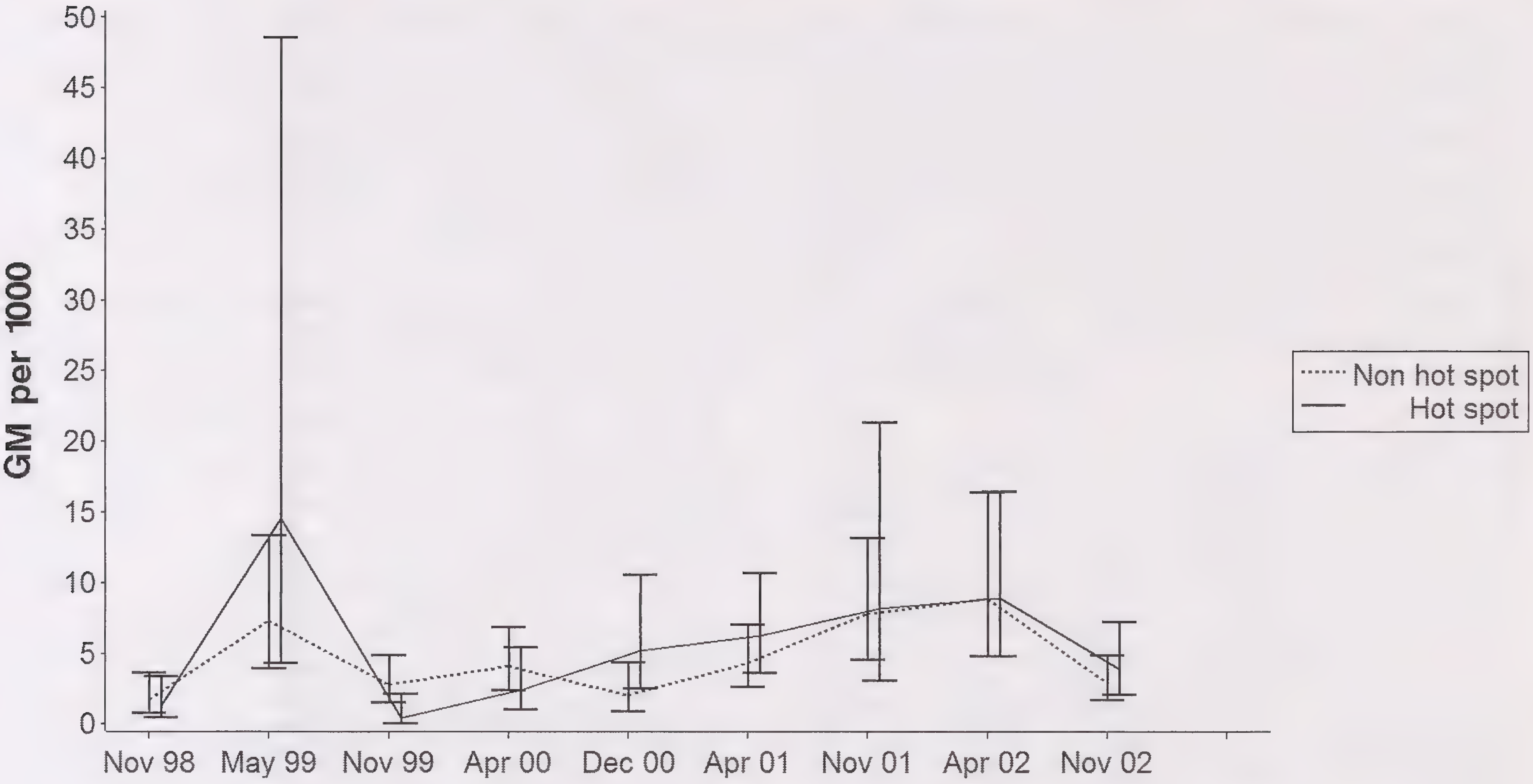

FIG. 15. Comparison of prevalence of infected snails in Hexi Village for hot spots vs. non hot spots employing geometric mean per 1,000 snails for nine seasons.

vs. non hot spots was much more pronounced than the density of infected snails. In Hexi (Fig. 15), the mean of means over nine seasons in non hot spots was 0.9 ± 0.97, whereas in hot spots it was 5.6 ± 4.88. The trend over the nine seasons was a sharp decease from season 2 to 3, then a regular increase from seasons 3 to 7 declining slightly to season 9. In Jishan (Fig. 16), the mean of means over nine seasons in non hot spots was 2.2 ± 0.3. Excluding the anomalous spike in season 2, the result was 0.3 ± 0.38. The prevalence was

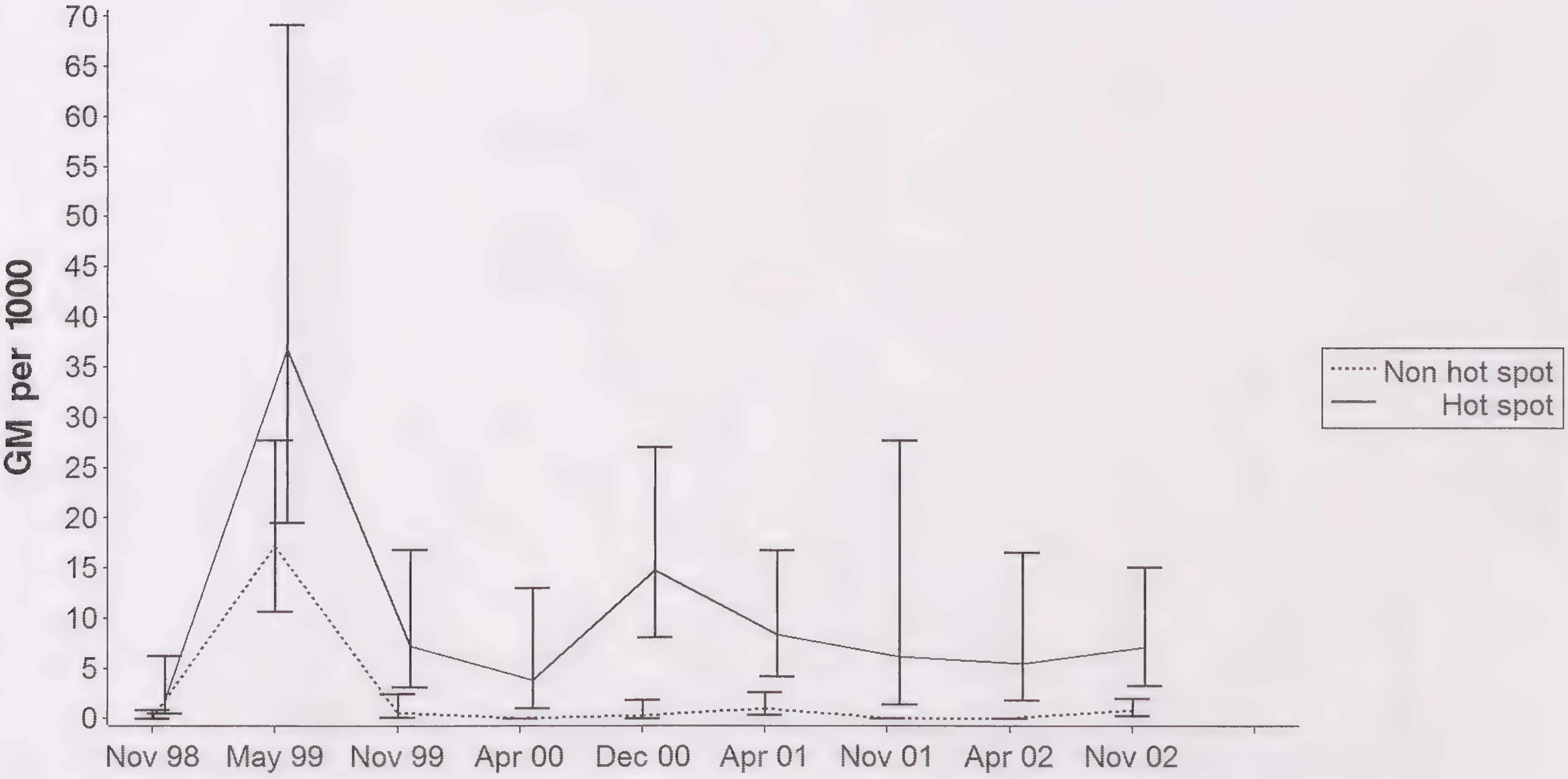

FIG. 16. Comparison of prevalence of infected snails in Jishan Village for hot spots vs. non hot spots employing geometric mean per 1,000 snails for nine seasons.

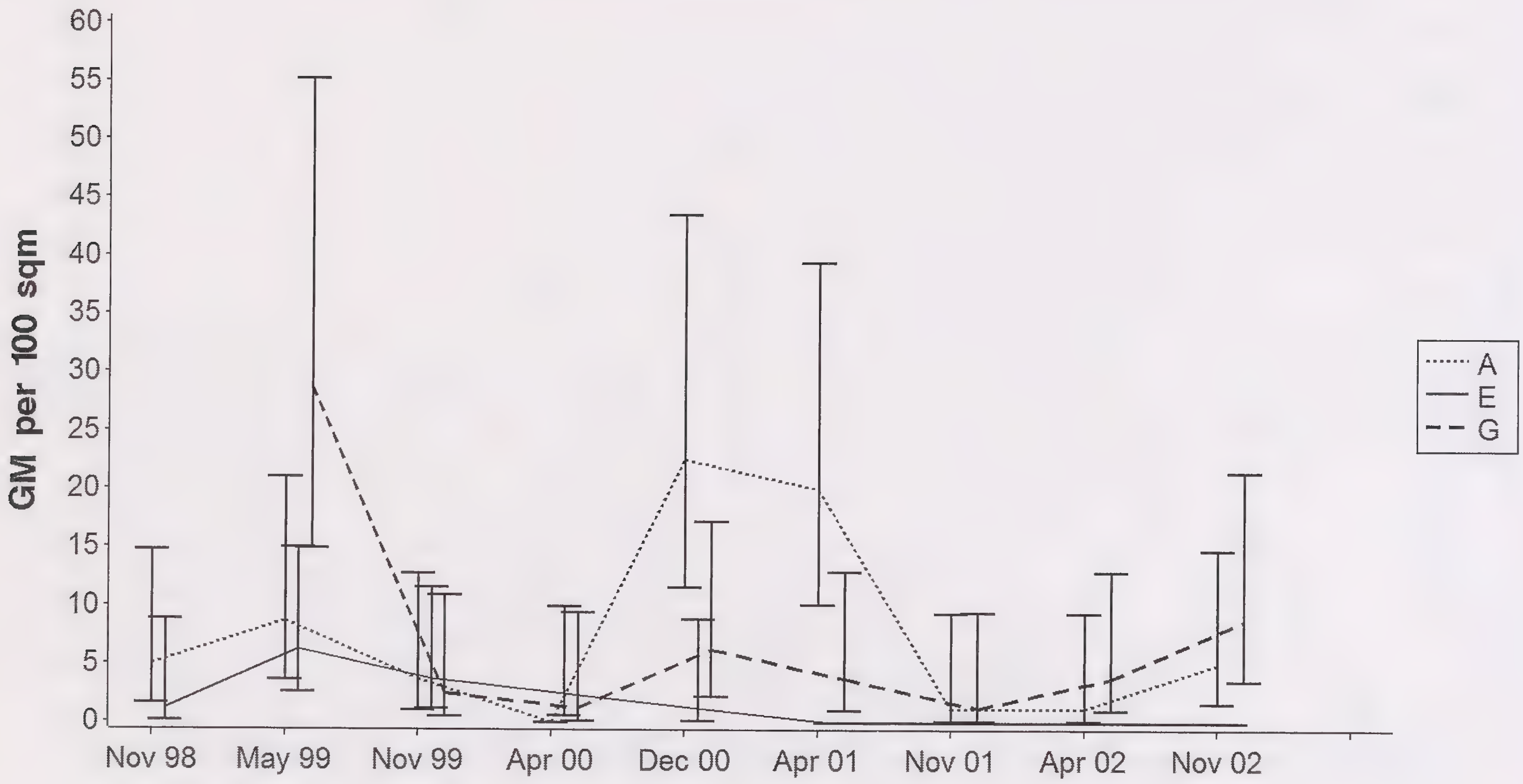

FIG. 17. Densities of infected snails in hot spots in Jishan Village: Village Zone (squares A, G) and Western Zone (square E) employing geometric mean of snails per 100 m^2.

considerably higher in hot spots. The mean of means over nine seasons was 7.5 ± 6.32. Excluding the spike in season 2, the result was 5.5 ± 2.2. As seen in Fig. 16, from season 3 onward the geometric mean of number of infected snails is not significantly different from 9/1,000. There was no net decrease over time.

Hot Spots in Zones – Density of Infected Snails

Jishan Village: The highest risks for gaining infections were in hot spots as seen by comparing the high risk Village and Western Zones with the low risk Southern Zone. There were two squares in the Southern Zone, but neither

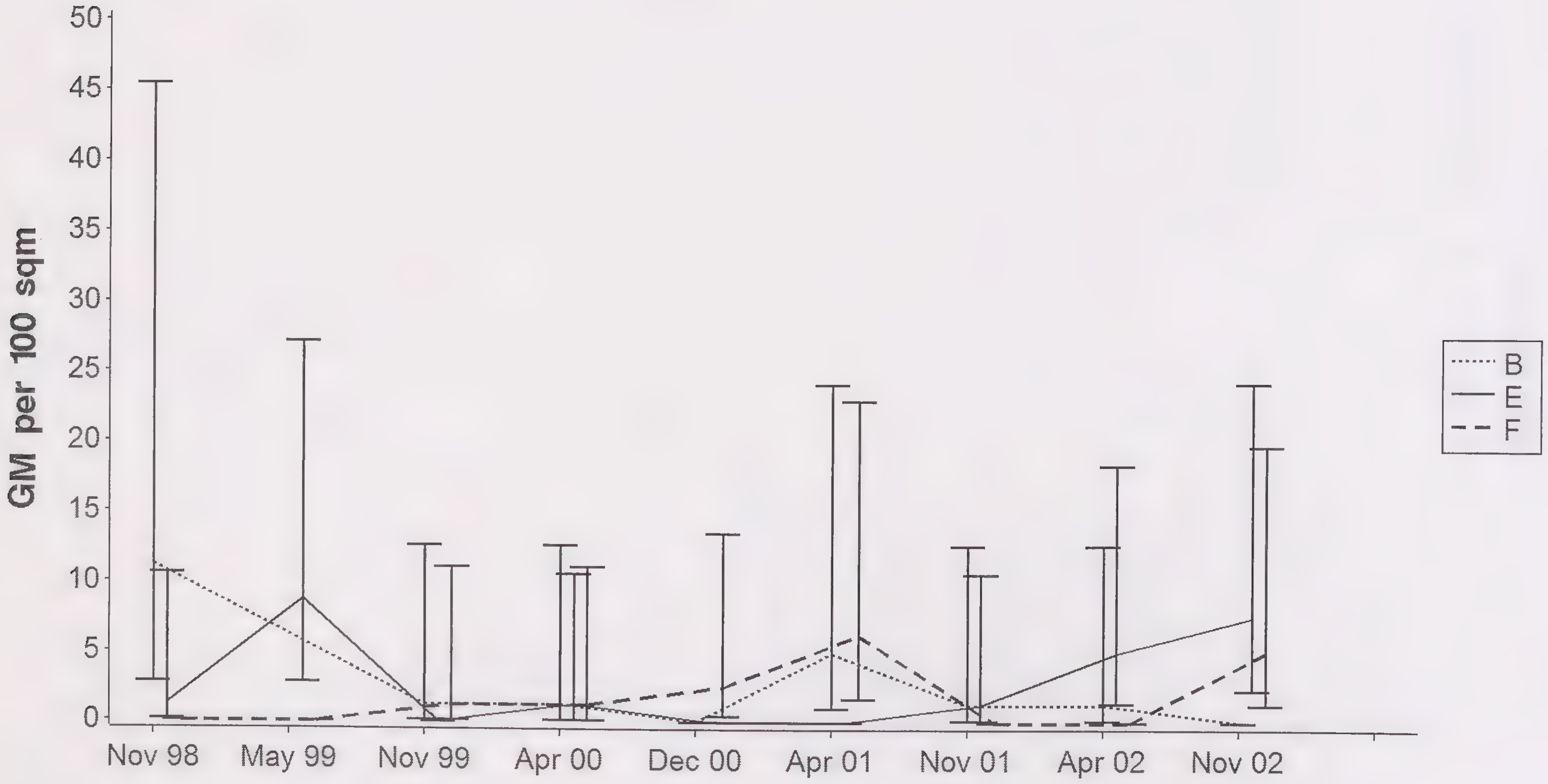

FIG. 18. Densities of infected snails in hot spots in Hexi Village: Shamo (square B) and Houshan (squares E, F) Zones employing the geometric mean per 100 m^2.

qualified as hot spots. Square C had 1 and 30 infected snails in seasons 1 and 2 respectively (= 1.47 and 19.44 infected snails per 100 m^2). Thereafter, no infected snails were collected. In the last season, after having no infected snails, one was found in square B (= 1.25 per m^2).

The most continuous substantial risks for infections were in the Village (sites A, G) and Western Zones (site E), but of these the zone closest to human and buffalo activity, the Village Zone, had the most severe continuous risks (Fig. 17). The density of infected snails in the Western Zone, after a marked increase in density and prevalence in season two gradually dropped to zero in the 6th season. However, in the Village Zone, the two hot spots had very different patterns of snail infections. In square A (Mexi Lake), the density of infections went from 11.7/100 m^2 in season 2 to zero in season 4, only to spike up to 25 and 22.2/100 m^2 in seasons 5 and 6 before dropping to levels of 5/100 m^2 or less in the last three seasons. In square G, very close to village houses, the pattern was quite different. In season 1 the site had not yet been discovered. In season 2, there was an extraordinary spike of infection (35.9/100 m^2). Density dropped in seasons 3 and 4 to levels not significantly different from levels seen in the other sites. Densities increased to over 5/100 m^2 in seasons 5 and 6, rising to 9.2/100 m^2 at the end of the study (the drop in season 7 seen in all sites was due to severe drought driving snails underground and thus uncollectible). For the Village Zone overall, statistically, the trends for these data are not significant comparing seasons 1, 2, 3, 4 to 9. There was a significant drop from season 5 to 9 attributed to the high spike in season 5 in square A (22% decrease, P = 0.021).The decrease from season 6 to 9 was not significant.

Hexi Village: There were two very different ecological zones. The east-of-plateau Shamo Zone equaled in every way the Southern Zone of Jishan in topography, high density of snails, density and abundance of grass and abundance of small ponds and pools in the "dry" season. Of three sites in Shamo, one hot spot (B) was found, a site closest to village daily activity. Due to flooding, it could not be collected in the second season; it had no infected snails in seasons 5 and 9. It had 11.25 infected snails/100 m^2 in season one and thereafter never exceeded 1.39/100 m^2. Analysis of trends over the nine seasons showed no significant differences (Fig. 18). Overall, in spite of this large area (Shamo) having high snail density, the density of infected snails was low to zero.

Houshan Zone was much more complex (Figs. 18, 19). Due to its size and irregular topography, six squares were originally chosen

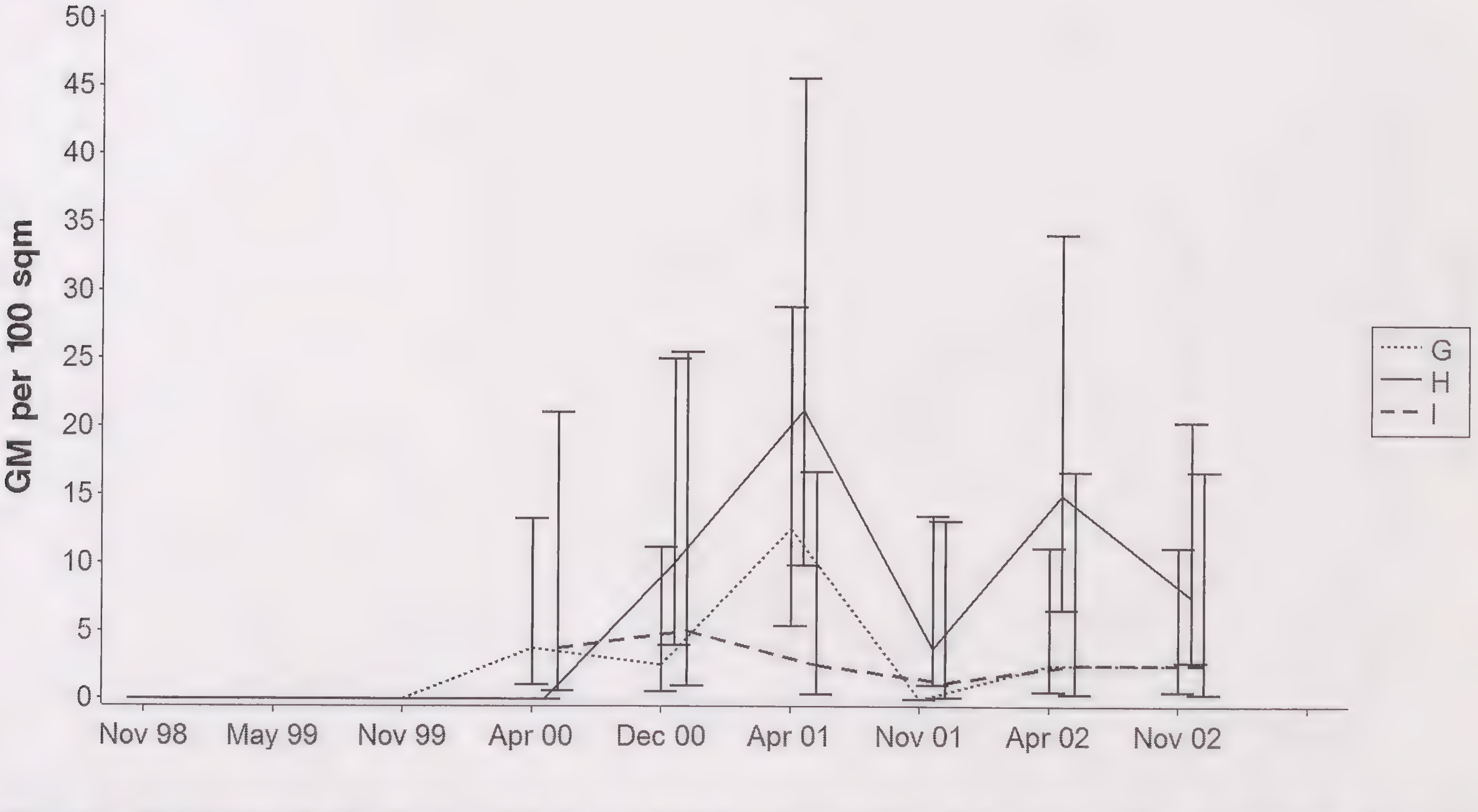

FIG. 19. Densities of infected snails in hot spots in Hexi Village: Houshan Zone (squares G, H, I) employing the geometric mean per 100 m^2.

with a 7th added in the 4th season, because a potential hot spot was discovered using the investigative technique of the "special collection" (see Methods). Of the seven sites (squares), five were hot spots. None went to zero infected snails for more than 3 or 4 seasons. Site G was flooded out one season and could not be collected; it was inadvertently not collected in the second season. Each hot spot was unique in pattern of density of infected snails. Only E had a significant spike of infection in the second season but not nearly as high as the unique spike in Jishan G (13.46/100 m^2 vs. 35.94 – three times higher). Four hot spots had elevated levels of infection in season 5 and 6 paralleling the increases seen in those seasons in Jishan A and G, but especially G.

As in Jishan, density of infected snails was low in season 7 due to the severe drought, and as in Jishan, increased in seasons 8 in E, G, H , and I. There was a significant increase at E in the last season. Taking Houshan hot spots as a whole, the trends of each season to season 9 were not significant except from season 8 to 9, when there was a significant 62.3% increase (P = 0.027). The parallel trend in Jishan (season 8 to 9) of 35% increase was not significant.

Comparing Hexi and Jishan: In Jishan, density in Village Zone site A started at 7.14 and was ≥ 5.0 in seasons 5, 6, and 9. At site G, the same held true in seasons 5, 6, 8 and 9. In the control village of Hexi, considering the critical seasons 5, 6, 8, and 9, and to make a comparison with the Jishan data above, hot spot B in Shamo had densities above the mean line of 3.54 infected snails/100 m^2 only in season 6, thereafter dropping below 1.50 and reaching zero in season 9. In Houshan sites F, G, and I, densities above 5.0/100 m^2 were attained in seasons 5 or 6 only to drop below 3.54 thereafter. There were increases above 3.54 in seasons 8 and 9 at sites E and H. In summary, in Hexi, there were only significant gains in density in seasons 8 and or 9 in just two hot spots, E and H.

Hot Spots in Zones- Prevalence of Infected Snails

Jishan: Prevalence data for hot spots are given in Fig. 20. As with density of infected snails, prevalence of infected snails had different dynamics in different hot spots. In the dominant at-risk Village Zone, there were two very different dynamics. At site A, season four had no infected snails and a spike to > 15 in season 5. Thereafter, the prevalence dropped to a level between two and four, not significantly different from what was found in season 1. In stark contrast, at site G, from season four onward the overall trend was from four infected snails per 1,000 to 40 in season 6,

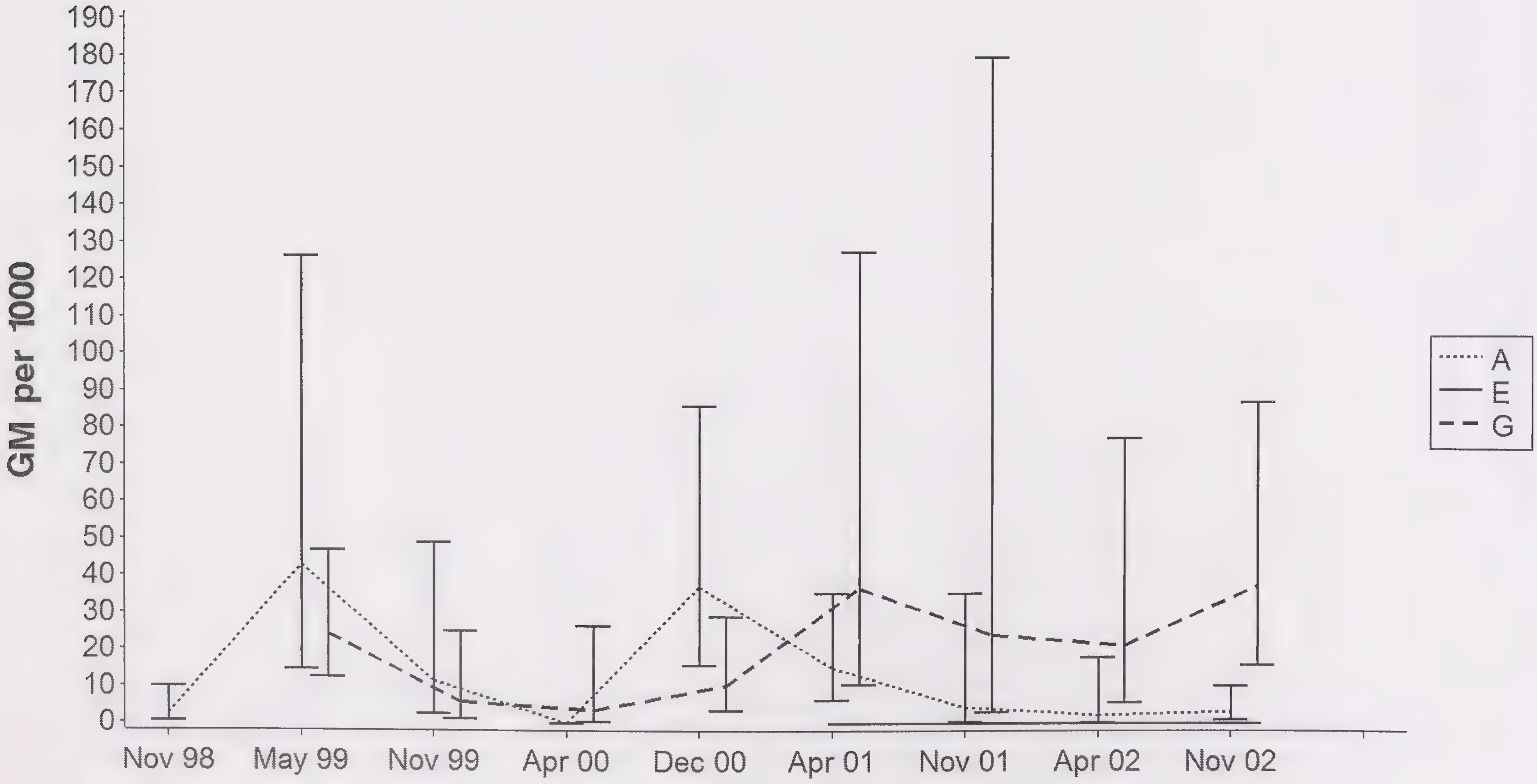

FIG. 20. Prevalence of infected snails in Jishan Village hot spots: Village Zone (squares A, G) and Western Zone (square E) employing the geometric mean per 1,000 snails.

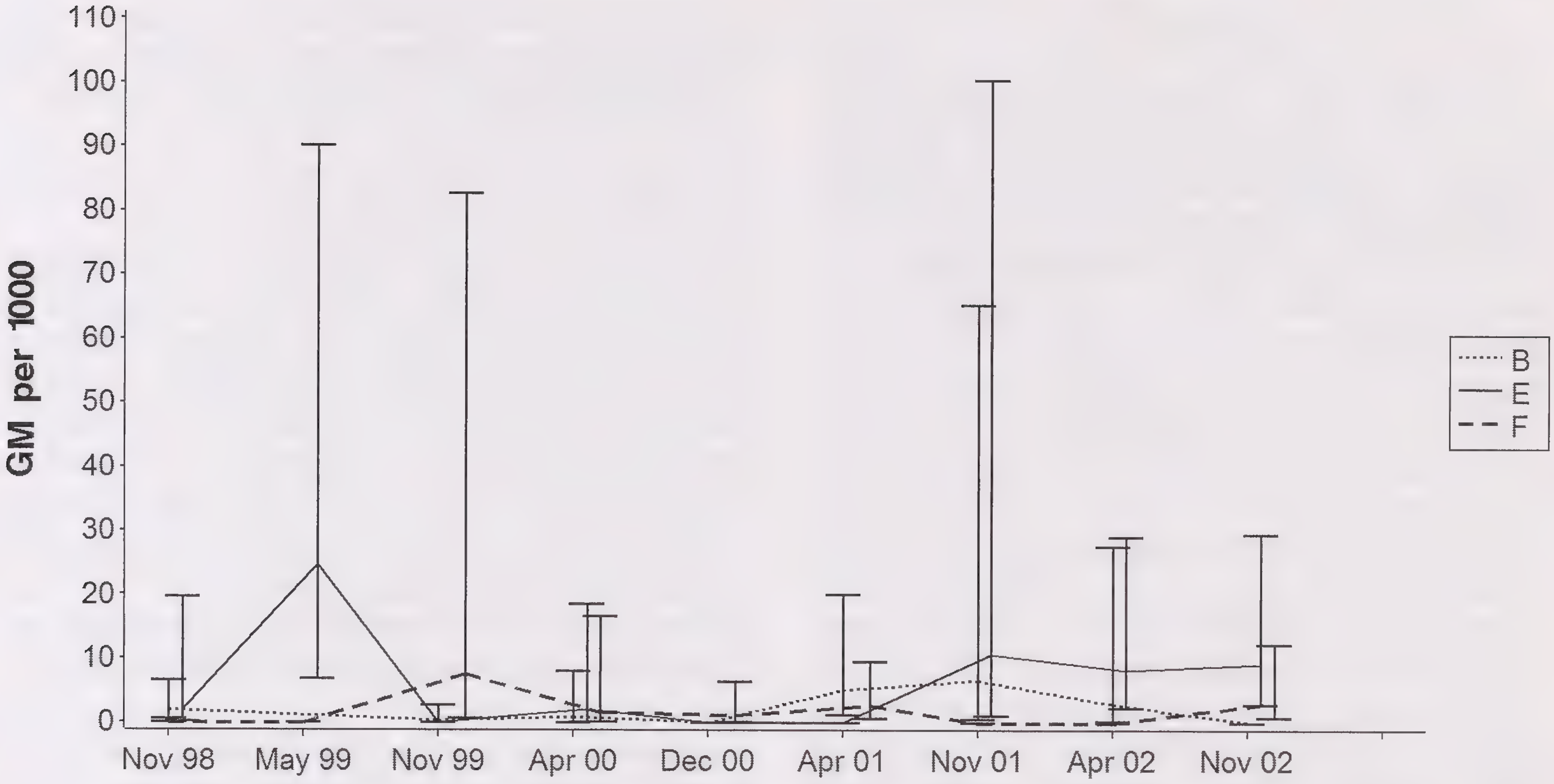

FIG. 21. Prevalence of infected snails in Hexi Village hot spots: Shamo Zone (square B) and Houshan Zone (squares E, F) employing the geometric mean per 1,000 snails.

decrease to 30 in season 8 and then a spike to 37 in season 9.

Hexi: Prevalence data for hot spots are given in Figs. 21 and 22. As in Jishan, there were very different dynamics among hot spots. Only Houshan E had a spike in prevalence of infected snails in season 2. From seasons 3 onward (post season 2 spike), there was a trend for significant increase by season nine only in Houshan sites E, and this a modest increase (< 10/1,000). Dramatic changes in prevalence were found at three Houshan sites: G, H, and I. From prevalence's ≥ 14/1,000, they all dropped to 6/1,000 or less in season 9. The only commonality among hot spots is a significant increase in prevalence in seasons 5 or 6, the same trend seen in Jishan.

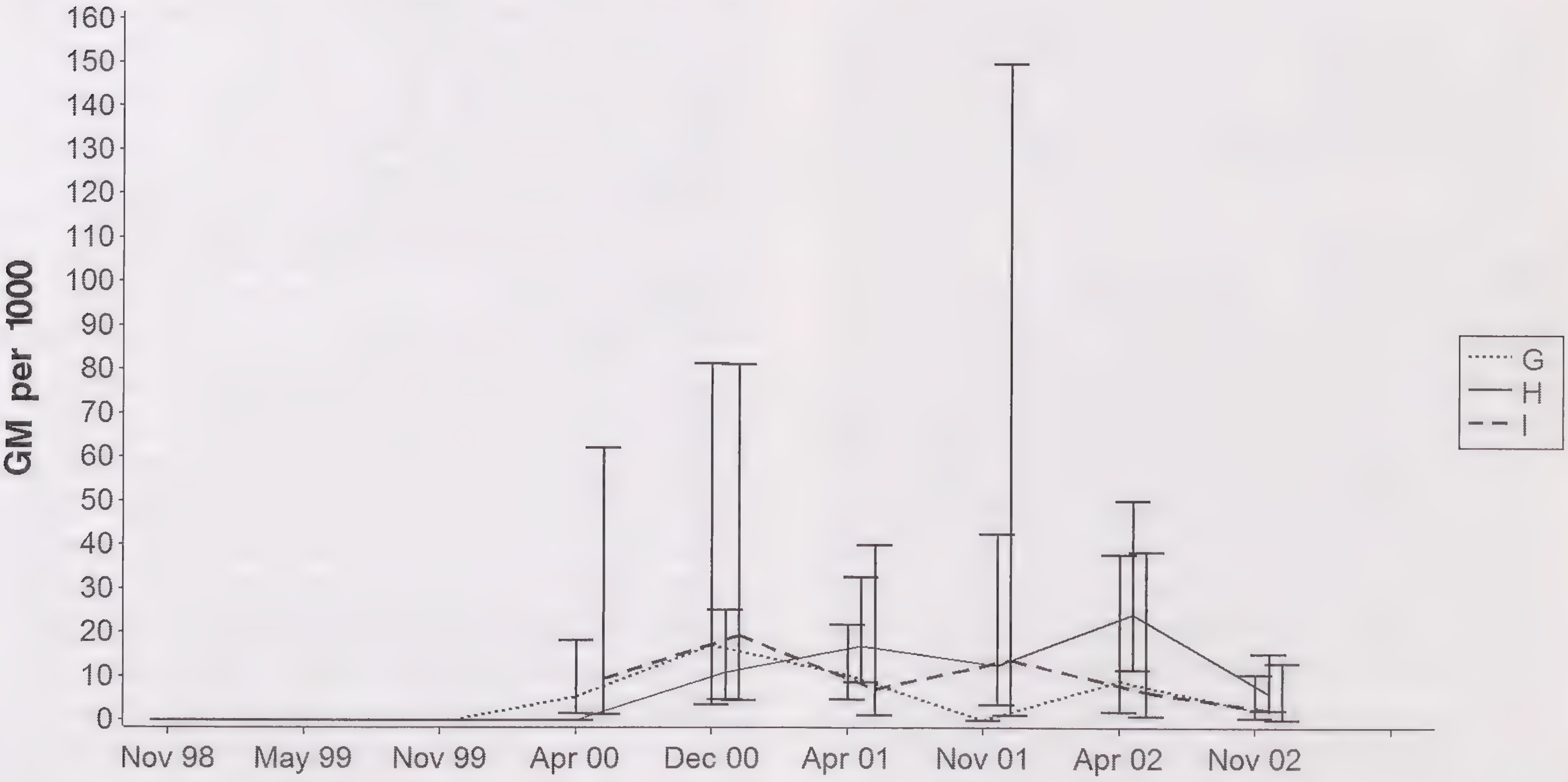

FIG. 22. Prevalence of infected snails in Hexi Village hot spots: Houshan Zone (squares G, H, I) employing the geometric mean per 1,000 snails.

DISCUSSION

The significant findings of this longitudinal study are several:(1) Foremost, the intervention did not achieve the goal of significantly reducing snail infections in Jishan. (2) To understand the dynamics of *Schistosoma japonicum* transmission throughout large areas such as the administrative villages of Hexi and Jishan, it is important to partition the area into clearly definable ecological zones. (3) We have found that the greatest risk of infection is in very small areas within zones that we call hot spots. (4) The most important indicator for risk of infection is the density of infected snails, not the prevalence. (5) There are very significantly more infected snails per area in hot spots than in non hot spots. (6) Density of infected snails is not correlated with density of snails overall. (7) The sampling strategy of zones, squares, and random selection of 20 4 m^2 cells with squares has enabled robust statistical analyses of snail populations that have a strong negative binomial distribution. Sampling twice a year (before the floods and after the floods) for at least 8 or 9 seasons has provided a time series enabling a robust assessment of trends in the experimental and control villages. Paying close attention to environmental factors as they impact results is critical. These findings will not necessarily be discussed in the order given above.

The Intervention

Theoretically, the treatment of people and water buffaloes in Jishan to rid them of infection should end the input of miricidia to snails. All infected snails could be expected to die within 12 to 18 months, and there would be no further source of infection. Over the nine seasons, with all sources of infections removed, schistosomiasis would be eliminated locally. Infected snails are the key! At the very least, one would expect a continuous decrease in density of infected snails in the experimental village compared to the control village. One should have at least an 80–90% reduction of infected snails. This did not occur. A quick look at Fig. 3 says it all at the village level. From season 3 onward the two villages did not differ significantly in density of infected snails (excepting season 8). There was no discernable trend towards < 0.5 infected snails per 100 m^2. There should have been no increases in infected snail density in seasons 5 and 6, more than half way through the experiment.

The probable reasons for the failure of the intervention are given at the end of the discussion after other factors are discussed.

Ecology, Environmental Factors, and Results

Snails must be sampled twice a year at the very least. The reasons for this are that these marshland ecosystems are dynamic with regard to variances in environmental factors. The vast marshland systems of Jiangxi and Hunan (Poyang and Dong Ting Lakes) are subjected to severe annual flooding. The floods coincide with the spring-summer monsoons. The timing and intensity of the monsoons vary. These combined with considerable season variance of temperature have a profound effect on snail populations.

Likewise, high temperatures and pronounced drought in summer severely affects snail populations. If the lakes flood early and the temperature is low, as in the severe floods of 1998, there will be one set of negative factors for snail survival and reproduction. If flood waters do not recede by late September, there will be other consequences. Late minimal flooding and early recession of floods (optimal conditions for snails) yields yet other results. All these factors relate to disease transmission. One cannot predict the timing of adverse affects on snail populations at either end of what is considered the "usual" flooding period.

In this study, it is clear that there is no clear seasonal bias on results. For example, one does not obtain consistently high numbers in the fall and low numbers in the spring. As the sampling technique does not enable picking up immature snails, the adults are collected in both seasons. Of note is that "old" adults are collected in both seasons, that is, a significant number of "older" adults survive the floods. So again, it is important to sample both sides of the flood period.

The extreme negative impact of the 1988 floods on snail populations, usually heavily infected, along the Gan River in Xinhua Village of Poyang Lake was documented (Davis et al., 2002a). The village flood plain was swept clear of snails by the extreme floods. In November 1988, about 90% of the entire area had no snails. Gradually snails re-colonized the area so that by December 2000 < 5% of the area had no snails. Snails per meter square in the one Xinhua hot spot increased from < 20 per frame in March 1999 to 72 by December 2000.

Given the example of Xinhua, it would appear that there was no adverse affect of flooding in the Jishan-Hexi Village marshlands. None of the Hexi-Jishan marshlands were swept clear of snails. In November 1988, there were, on average, > 65 snails per frame in both villages (no significant difference), and at the end of the experiment there were > 50 snails per frame (no significant difference between villages). Only in season two (May 1999) was there a significant drop in density in both villages to 15–20 per frame (Jishan had significantly more), levels similar to those seen in season 8. Accordingly, one cannot attribute the infection data to flood related decrease in snail density at the beginning of the experiment.

There was apparently a flood-related impact on the percentage of area that had no snails as seen in Fig. 1. Three zones had > 25% area with no snails from seasons 1–4 that decreased to 6–21% in seasons 5–9. The zones with the highest snail densities (Jishan-Southern and Hexi-Shamo) did not have such large percentages of land without snails throughout the experiment. As more area obtained snails in the zones indicated, there is no evidence that this impacted overall snail density or density of infected snails. The snails simply expanded into previously vacated habitat.

Anomalous Results

There are two results that are considered anomalous in this experiment. One can be explained on the basis of ecological observations made during each sampling period. There is, as yet, no explanation for the other. The explainable anomaly is the season 7 low numbers in all data. These low numbers in Jishan are not the result of the intervention. The same low numbers were found in Hexi. There was a sever drought on the marshlands in the late summer and fall of 2001. With the prolonged absence of water the snails go underground and aestivate. Sampling during this period yielded numbers that considerably underestimates the actual number of snails present. To illustrate this point, one of us (GMD) went to collect snails from Lao Zhou Island, Anhui, in June some years ago. The island was available then for the very reason that the monsoons were delayed and there had been a summer drought. During the monsoons the island flood plains are under water. The morning collection of *Oncomelania* by some 15 persons yielded a negligible number of snails (< 2 snails/m^2). During the end of the collection period, it began to rain. We left the area and returned three hours later, during which time the rain was continuous and heavy. We again collected the same area and obtained a high density yield (> 28 snails/m sq). With the available moisture soaking the ground the snails came to the surface to resume activities associated with an optimal habitat. The sampling problem seen above is one encountered by all standard collecting procedures used. The caution is to attempt to collect during periods of normal soil moisture associated with active snail behavior.

The other anomalous result was the exceptional spike of infected snails in season two in Jishan. The 19.5 infected snails per 100 m^2 for Jishan Village as a whole is far above the data recorded for the lake region. To our knowledge, it is a unique occurrence. The historical record for this quadrant of the lake averages 2 per 100 m^2 (Anonymous, 1985; Davis et al., 2002). The spike is primarily a phenomenon of hot spot G in Jishan where the number > 35/100 m^2. The only plausible explanation is that one or more heavily infected buffaloes defecated directly on this site several times over a period of time infecting numerous snails in the restricted area. It occurs when the density of snails actually had a significant decrease.

If one ignored these anomalies, and used ANOVAS to only examine trends from season one to season nine, one would come to an erroneous conclusion. For example, as there is a significant decrease in infected snails from season one to season 9 (15%; P = 0.00007) one might be tempted to say that the intervention was working. This clearly was not the real situation as discussed above.

Zones, Snail Density, Snail Infections, and Hot Spots

Remote sensing using Landsat TM images has enabled classification of the these marshlands into three general types: (1) mud flats grading into areas supporting some grass; (2) cattle grazing range that grades from water-covered grass extending throughout the marshlands that support grasses of all types, including higher dryer areas that can still support grass; and (3) snail habitat where the land within the larger cattle grazing range is both dry enough and moist enough to support snails (Davis et al., 2002b).

In this study, areas that were capable of supporting snails were divided into ecological zones. The results show the value of partitioning the villages into ecologically discernable zones. Two zones, Jishan-Southern and Hexi-Shamao, had the most ideal snail habitat over a vast area and supported the greatest density of snails, generally over 60 snails per frame (15/m^2; medium to high density, Table 2, Davis et al., 2002a). In Jishan, the Village Zone was less than optimal snail habitat due to the fact that in recent years rising lake levels were forcing the conversion of that zone from farming fields to marshland. The zone did not have the vast level land at an elevation above mean low water supporting lush growths of thick, tall grasses that provide the shade and humidity most suitable for *Oncomelania.* The Western Zone of Jishan had large areas much too wet for optimal *Oncomelania* habitat. In this zone grasses were frequently partially to wholly covered by water for prolonged periods of time. In these two less than optimal zones snail density was generally < 35 snails/frame (8.5/m^2; low density). For reasons we cannot explain, in the last three seasons (excluding the dry seventh season), the snail density in Shamo dropped to levels insignificantly different from those on the Houshan Zone.

The Houshan-Zone of Hexi was one dissected by ingressions from the lake and large areas sloping up to elevations marginal for snails. Overall, the places most suitable for snails were restricted into narrow strips of land supporting rich stands of grass bordering the ingressions from the lake. Over the first five seasons the density was low. In the remaining seasons (excluding the dry period of season 7) the density increased to about 45/frame (11/m^2; medium density).

It is counter-intuitive that the snail dense zones do not have the highest density of infected snails, but that is the situation. This was noted before (Davis et al., 2002b) for Hexi and Jishan. The correlation between snail density and prevalence of infection for all sites in Hexi, after seven seasons, was r = -0.189; in Jishan r = -0.210. Considering all nine seasons for both villages, the correlation between snail density and density of infected snails was r = -0.134. Highest density areas for snails such as Shamo in Hexi and the Southern Zone in Jishan have the lowest density of infected snails. In Hexi- Shamo, the mean of means for snails/frame was 79; infected snails per 100 m^2 were 1.4. The contrast with the Houshan Zone was 22 snails per frame and 2.3 infected snails per 100 m^2. In Jishan, the mean of means for snails/frame in the Village Zone was 23; infected snails per 100 m^2, 3.8. The contrast with the Southern Zone was 84 mean number of snails/frame. Infections dropped to zero after season two. In seasons 1 and 9 (excluding the anomalous spike in season two) the infected snails/100 m^2 averaged below 2.1.

Hot spots account for the high density of infected snails in areas otherwise with low density snails. It is informative that in snail rich Shamo, only one of six hot spots was maintained and this a rather weak one. As shown, densities of infected snails in hot spots were considerably higher than densities in non hot spots. In Hexi, infected snail densities in hot spots averaged 3.5 ± 4.5/100 m^2; in Jishan, excluding the season 2 anomaly, it was 5.73 ± 6.76/100 m^2 (A, G, and E until E dropped to zero). The hot spots are relatively close to centers of activity, such as natural village dwellings and areas where human activity, buffalo convergence zones (in their daily patterns of leaving the villages to forage and returning home in the evening), and suitable snail habitat occurs. There is a strong correlation between hot spots and the short distance from active sites mentioned above (r = > 0.80; Davis et al., 2002a). These hot spots are not large, averaging about 30,000 m^2 each (N = 9; Davis et al., 2002a). What is important in hot spots is not the density of snails, but a site generally favorable for snails each year where sufficient buffaloes defecate regularly, depositing thousands of *S. japonicum* eggs, so that numbers of snails become infected relatively frequently. Given the right circumstances, even a marginal snail habitat can be a hot spot.

Density of Infected Snails vs. Prevalence

It is clear that the most important indicator of risk of infection is how many snails are infected per area, not how many snails are infected per 100 or 1,000 snails, that is, prevalence. Prevalence of infected snails is not correlated with density of snails ($R^2 = 0.16$ in Jishan, 0.013 in Hexi). Prevalence can vary wildly in relationship to density of infected snails as seen in Fig. 23. Over 75% of snails infected per 1,000 snails occur where the density of infected snails is ≤ 6 snails per 100 m^2; 55% of the snails infected per 1,000 snails occur where the density of infected snails is ≤ 3 infected snails per 100 m^2. The spread of the number of infected snails per 1,000 snails is considerable, ranging from < 1 to 23 where the density of infected snails was one per 100 m^2. The range was three to 47 at 6 infected snails per 100 m^2.

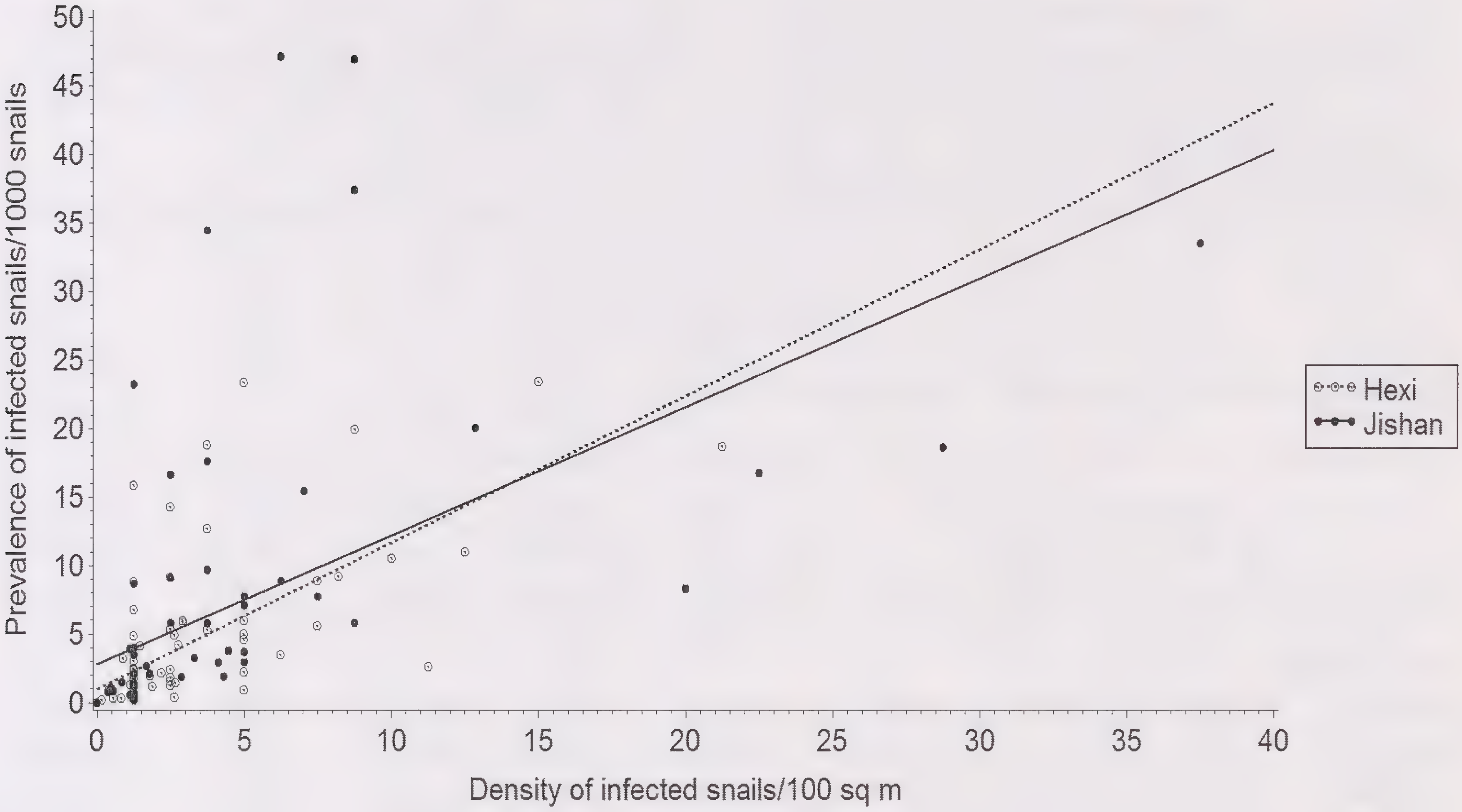

FIG. 23. Plot of density of infected snails x prevalence of infected snails for Hexi and Jishan Villages.

Probable Reasons for Intervention Failure

There are two probable reasons why snail infection rates were not decreased by the mid or end of the experiment. First, buffaloes were examined and treated only once a year. During the post treatment year buffaloes could be reinfected as infections are possible in both pre- and post-flood periods as long as the temperature does not go to 10°C or below, causing the snails to become inactive. Second, pregnant females were not treated. As the majority of the buffaloes herd was female and of reproductive age and included pregnant individuals, there was no complete control of the herd for eliminating infections. It only takes one or two infected buffalo defecating in hot spots to maintain the steady state density of infected snails over the nine seasons. Buffaloes fecal patties are immense and can contain thousands of eggs. Hot spots, as discussed above, are areas that are close to human habitation, where there is maximal and daily visitation by man and buffaloes in an environment continuously supporting snails, even if the snail density is low. Other animals can be discounted as a source of infection. Pigs would be the most likely candidates, but they were always penned. There were no dogs, goats or sheep.

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APPENDIX 1

Total snail data for two villages. *Arithmetic mean.

	All Snails				Infected Snails			
Season	No. of Frames	Total No. of Snails	Mean*	No./m^2 Mean*	No./4 m^2 Frames	Mean*	Per m^2 Mean*	No. of Snails
Hexi Village								
Nov-98	159	10,011	62.96	15.74	12	0.075	0.019	0.0012
May-99	120	1,677	13.98	3.5	7	0.058	0.015	0.0042
Nov-99	140	8,676	61.97	15.49	3	0.021	0.005	0.0003
Apr-00	200	6,582	32.91	8.23	9	0.045	0.011	0.0014
Dec-00	200	10,797	53.99	13.5	20	0.1	0.025	0.0019
Apr-01	200	8,641	43.21	10.8	40	0.2	0.05	0.0046
Nov-01	200	2,174	10.87	2.72	7	0.035	0.009	0.0032
Apr-02	180	3,496	19.42	4.86	21	0.117	0.029	0.006
Nov-02	200	10,631	53.16	13.29	20	0.1	0.025	0.0019
Jishan Village								
Nov-98	119	9,520	80.00	20.0	6	0.05	0.013	0.0006
May-99	140	3,585	25.61	6.4	72	0.514	0.129	0.0201
Nov-99	140	4,692	33.51	8.38	10	0.071	0.018	0.0021
Apr-00	140	3,432	24.51	6.13	3	0.021	0.005	0.0009
Dec-00	140	6,545	46.75	11.69	25	0.179	0.045	0.0038
Apr-01	140	7,800	55.71	13.93	23	0.164	0.041	0.0029
Nov-01	140	2,477	17.69	4.42	2	0.014	0.004	0.0008
Apr-02	120	2,653	22.11	5.53	4	0.033	0.008	0.0015
Nov-02	140	8,440	60.29	15.07	16	0.114	0.029	0.0019

APPENDIX 2

Zones. All snail data.

	All Snails				Infected Snails			
Season	No. of Frames	Total No. of Snails	Mean/ Frame	Mean/ m^2	Total No. of Snails	Mean/ Frames	Mean/ m^2	Prevalence Infected Snails
Hexi Village								
Shamo Zone								
Nov-98	39	8,350	214.1	53.53	11	0.282	0.071	0.0013
May-99	20	1,179	58.95	14.74	0	0	0	0
Nov-99	40	5,653	141.3	35.33	1	0.025	0.006	0.0002
Apr-00	60	3,639	60.65	15.16	1	0.017	0.004	0.0003

(continues)

(continued)

	All Snails				Infected Snails			
Season	No. of Frames	Total No. of Snails	Mean/ Frame	Mean/ m^2	Total No. of Snails	Mean/ Frames	Mean/ m^2	Prevalence Infected Snails
Dec-00	60	6,455	107.58	26.9	4	0.067	0.017	0.0006
Apr-01	60	2,215	36.92	9.23	4	0.067	0.017	0.0018
Nov-01	60	1,076	17.93	4.48	1	0.017	0.004	0.0009
Apr-02	60	1,153	19.22	4.81	1	0.017	0.004	0.0009
Nov-02	60	3,253	54.22	13.56	0	0	0	0
Houshan Zone								
Nov-98	120	1,661	13.48	3.46	1	0.008	0.002	0.0006
May-99	100	498	4.98	1.25	7	0.07	0.018	0.0141
Nov-99	100	3,023	30.23	7.56	2	0.02	0.005	0.0007
Apr-00	140	2,943	21.02	5.26	8	0.057	0.014	0.0027
Dec-00	140	4,342	31.01	7.75	16	0.114	0.029	0.0037
Apr-01	140	6,426	45.9	11.48	36	0.257	0.064	0.0056
Nov-01	140	1,098	7.84	1.96	6	0.043	0.011	0.0055
Apr-02	140	2,343	19.53	4.88	20	0.167	0.042	0.0085
Nov-02	140	7,378	52.7	13.18	20	0.143	0.036	0.0027
Jishan Village								
Village Zone								
Nov-98	40	1,128	28.2	7.05	4	0.1	0.025	0.0035
May-99	60	1,383	23.05	5.76	30	0.05	0.125	0.0217
Nov-99	60	1,193	19.88	4.97	5	0.083	0.021	0.0042
Apr-00	60	784	13.07	3.27	1	0.017	0.004	0.0013
Dec-00	60	1,757	29.28	7.32	23	0.383	0.096	0.0131
Apr-01	60	2,517	41.95	10.49	23	0.383	0.096	0.0091
Nov-01	60	324	5.4	1.35	2	0.033	0.008	0.0062
Apr-02	60	717	11.95	2.99	4	0.067	0.017	0.0056
Nov-02	60	2,093	34.88	8.72	15	0.25	0.063	0.0072
Southern Zone								
Nov-98	40	7,692	192.3	48.08	1	0.025	0.006	0.0001
May-99	40	2,094	52.35	13.09	37	0.925	0.231	0.0177
Nov-99	40	2,864	71.6	17.9	0	0	0	0
Apr-00	40	2,311	57.78	14.45	0	0	0	0
Dec-00	40	3,855	96.38	24.1	0	0	0	0
Apr-01	40	3,239	80.98	20.25	0	0	0	0
Nov-01	40	1,504	37.6	9.4	0	0	0	0
Apr-02	40	1,724	43.1	10.78	0	0	0	0
Nov-02	40	4,909	122.7	30.68	1	0.025	0.006	0.0002
Western Zone								
Nov-98	39	700	17.95	4.49	1	0.026	0.007	0.0014
May-99	40	108	2.7	0.68	5	0.125	0.031	0.0463
Nov-99	40	635	15.88	3.97	5	0.125	0.031	0.0079
Apr-00	40	337	8.43	2.11	2	0.05	0.013	0.0059
Dec-00	40	933	23.33	5.83	2	0.05	0.013	0.0021
Apr-01	40	2,044	51.1	12.78	0	0	0	0
Nov-01	40	649	16.23	4.06	0	0	0	0
Apr-02	20	649	16.23	4.06	0	0	0	0
Nov-02	20	212	10.6	2.65	0	0	0	0

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THE BIOLOGY AND ECOLOGY OF THE GIANT FREE EGG CAPSULES OF *ADELOMELON BRASILIANA* LAMARCK, 1811 (GASTROPODA: VOLUTIDAE)

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ABSTRACT

A three-year study was conducted to determine oviposition periods of the volutid gastropod *Adelomelon brasiliana,* the abundance and distribution pattern of its egg capsules in the region of Mar del Plata, Argentina. Results indicate that oviposition was correlated with water temperature. Reproduction occurred from September/October to May/June. Because egg capsules are not attached to the substratum and drift freely on the bottom along a narrow zone close to the shoreline, they can become stranded on the beach after storms, thus suffering mass mortality. This was reflected by a decline in snail recruitment during one study year in which storm surges were frequent and severe. The developmental stages of the egg capsules were characterized, and the proportion of early and late developmental stages determined monthly. This allowed an estimate of recruitment during each reproductive season. Twenty percent of the egg capsules at hatching contained one to three embryos that were considerably smaller than their siblings and 6% of the egg capsules at the same developed stage had one to four teratological embryos. Egg capsules laid down on the sea bottom showed an aggregated distribution pattern. The average developmental time was 57 ± 4 days. Protein and sugar concentration and pH of the intracapsular fluid decreased as embryo development progressed. Several proteins with different molecular weights were present in the intracapsular fluid during the entire intracapsular development.

Key words: *Adelomelon brasiliana*, Volutidae, oviposition, distribution pattern, storm events, intracapsular development stages, reproductive season.

INTRODUCTION

The Family Volutidae is widely distributed being found in the Indo-Pacific Ocean as well as along the coasts of Africa, Antarctica, Central and South America (Poppe & Goto, 1992; Abbott, 1974; Abbott & Dance, 1986).

Recent species belonging to this family undergo intracapsular embryonic development and hatch as crawling juveniles (Penchaszadeh et al., 1999). Volutids from the Indo-Pacific Ocean deposit pineapple-like egg mass containing several egg capsules. In most cases, a single embryo develops within each egg capsule. The egg masses are attached to rocky substrata or to empty bivalve shells, as is the case of species of the genus *Melo* (Allan & Middleton, 1946; Knudsen, 1993; Cotton, 1936).

In volutids from the African Atlantic Ocean of the genus *Cymbium*, egg capsules are brooded in a special "pouch" in the foot, and although egg capsules contain several embryos, only one of these attains complete development (Marche-Marchad, 1968).

South American and Caribbean species differ from those of the African and Indo-Pacific groups in that development occurs within egg capsules containing a variable number of embryos without nutritive nurse eggs, except for *Voluta virescens* Lighfoot, 1786, in which one or two embryos are present at hatching (Bandel, 1976). In general, American egg capsules are almost semi-spherical and are attached to hard substrata by a flat base (Penchaszadeh et al., 1999).

Adelomelon brasiliana, Lamarck, 1811, is a common species in the southwestern Atlantic. It inhabits shallow sandy bottoms from Rio Grande do Sul, Brazil, to San Antonio Oeste, Argentina. Published studies of this species include its anatomy (Novelli & Novelli, 1982), gonadal cycle (Cledón et al., 2005a), individual growth (Cledón et al., 2005b), and the association with the sea anemone *Antholoba achates* (Drayton, in Dana, 1846) (Luzzatto & Pastorino, 2006).

Egg capsules of *Adelomelon brasiliana* are an exception to the description given above for the South American group because they are laid freely on the sea bottom without being attached to any substratum. This is a unique characteristic among volutid species for which egg capsules have been studied. *Adelomelon brasiliana* egg capsules were first described by d'Orbigny (1846), and the earliest studies on the embryology and the intracapsular liquid were conducted by de Mahieu et al. (1974).

Considering volutids from the Atlantic Ocean, egg capsules are known for the following species: *Zidona dufresnei* (Donovan, 1823), *Odontocymbiola magellanica* (Gmelin, 1791), *Adelomelon beckii* (Broderip, 1836), *Adelomelon ancilla* (Lighfoot, 1786), and *Voluta musica* Linnaeus, 1758 (Penchaszadeh & de Mahieu 1976; Penchaszadeh, 1988; Penchaszadeh et al., 1999; Penchaszadeh & Miloslavich, 2001).

Previous studies on *Adelomelon brasiliana* and *Voluta musica* pointed out an important energetic investment in reproduction, as indicated by the high concentrations of proteins and carbohydrates in the intracapsular liquid, making possible a complete embryonic development within the egg capsule (de Mahieu et al., 1974; Penchaszadeh & Miloslavich, 2001). Such an important investment is also expected for such other volutid species as *Adelomelon ancilla*, *Adelomelon beckii*, *Odontocymbiola magellanica*, and *Zidona dufresnei,* because of their similarity in the reproductive mode (Penchaszadeh et al., 1999).

This study focuses on the embryonic development, developmental time, seasonal oviposition, distribution pattern and intracapsular fluid content of *Adelomelon brasiliana* egg capsules. I also examined the potential effects of inshore drift currents increased by southeast winds, which result in storm surges and unusual swells. All these parameters are relevant in the understanding of this reproductive mode in an environmental context.

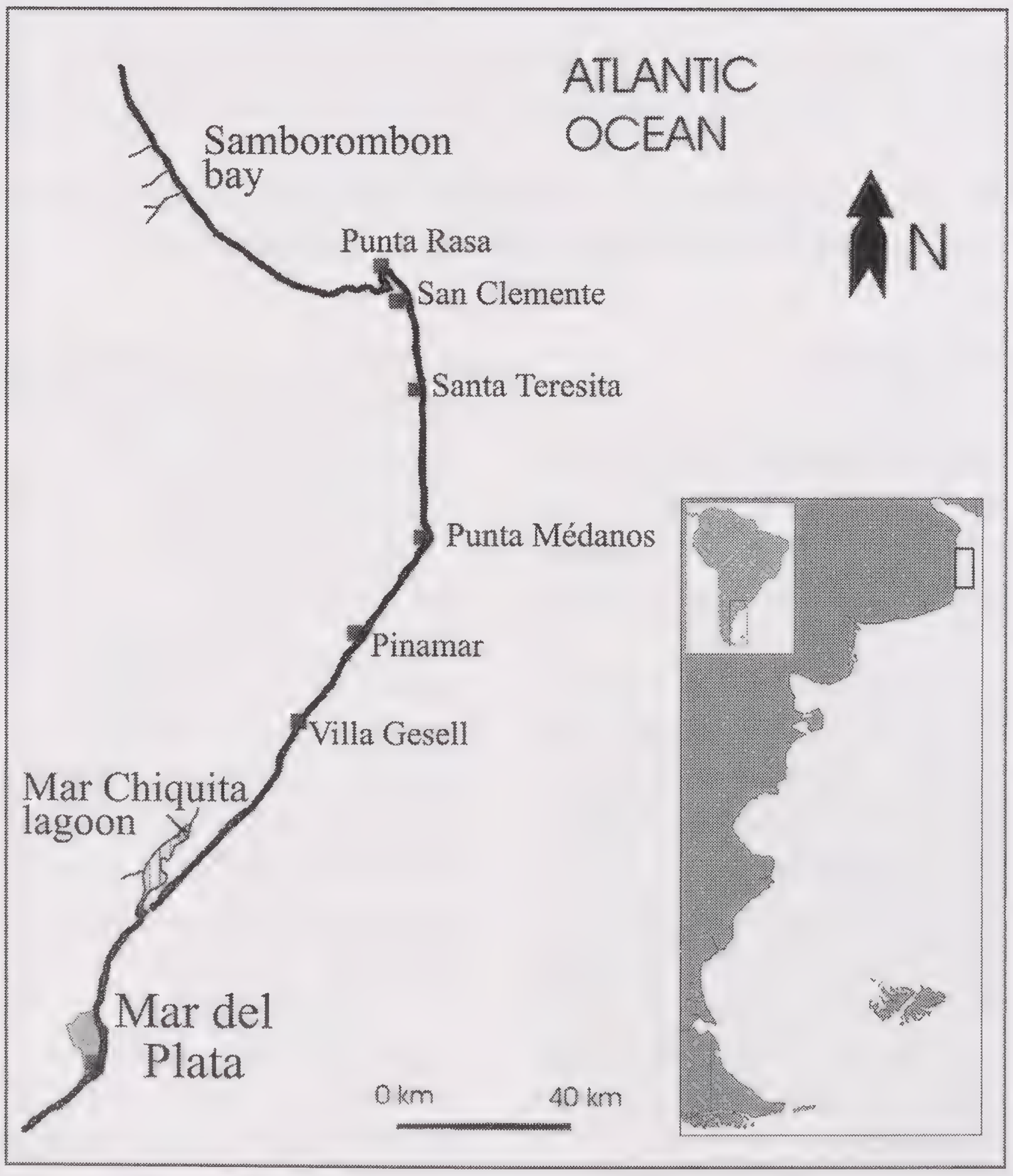

FIG. 1. Geographic position of the sampling area and the most important adjoining localities.

MATERIALS AND METHODS

Samples were taken monthly from April 2000 to March 2003 near the outlet of the Mar del Plata harbor (~38°S) (Fig. 1). Egg capsules were collected with a beam trawl net 1 m wide, 1 cm mesh, along line transects parallel to the shoreline at depths between 8 and 20 m. Each monthly survey consisted of three trawls, which lasted for 15 min each. Taking into account the direction and strength of the current and dominant winds during the trawl operation, the estimated area covered by the net in each event ranged from 900 to 1,500 m^2. Density was expressed as number of egg capsules per 100 m^2.

To determine the distribution pattern of egg capsules on the sea bottom, an additional survey was conducted using the same boat and net as before in 12 randomly chosen sampling sites in the same study area on December 18, 2002. One sample was taken for 10 min at each site. The mean number and SD of all egg capsules obtained from the 12 trawls were calculated.

The external and internal volumes were recorded from 143 egg capsules. The external volume was measured indirectly by the displaced volume using a 500 ml test tube. The intracapsular fluid was extracted and measured with a test tube for the internal volume determinations. A linear regression was performed between the two measures. The volume occupied by the embryos was not considered because in an advanced developmental stage embryos represent less than 5% of the intracapsular volume.

The number and size of embryos per egg capsule were recorded, and the developmental stage of embryos determined following the scale established by de Mahieu et al. (1974). One-way ANOVA was used to determine differences between the number of embryos per egg capsule and their developmental stage. The relationship between the number of embryos and the egg capsule volume was analysed using linear regression.

Mean monthly temperatures of surface water (1 m) and their respective SD were calculated from daily records provided by the Mar del Plata harbor marigraph. The correlation between the mean monthly temperatures of surface water and presence of egg capsules on the sea bottom was made using Kendal's Method (Conover, 1999). This non-parametric statistical method was applied because data did not meet assumptions of normality and homoscedasticity.

During the study period, all storm events taking place in the Partido de la Costa were registered (San Clemente del Tuyú marigraph; López, pers. com.). These were referred to as the number of days with waves exceeding 2.1 m.

The total embryonic developmental time and the developmental time of each stage were determined in aquaria under controlled conditions of salinity (35‰) and temperature (ranging between 18 and 24°C). In the experiment, 30 egg capsules at a very early developmental stage were placed in each of two 200-liter tanks. Seawater was recycled through a refrigerator with an aquarium water pump. Every 2 days, egg capsules were removed from the water for a short period to determine their individual developmental stage (transparency enabled direct scoring) and returned to the aquaria. Aquaria were equipped with a pump for water movement because preliminary experiments had shown that this was a crucial requirement for the viability of embryos in egg capsules at early developmental stage (lower than stage 2).

Recruitment was expressed as the percentage of the number of egg capsules that hatched or were about to hatch (stages 5 to 8), using all egg capsules collected during each reproductive season.

Two ml of intracapsular fluid of 40 egg capsules at different developmental stages were frozen and used for chemical analysis. Protein concentrations were determined using the Lowry method using BSA to build the calibration curve. Total carbohydrate was determined by the Herbert et al. (1971) method. Analytical glucose was used for the calibration curve. After dissecting the living egg capsules, pH was measured at different developmental stages. SDS-page electrophoresis (gels containing 10% acrylamide) was carried out using 3 µg of proteins per lane of the intracapsular fluid previously determined. Biorad prestained standards were used. The gels were stained first with Biorad silver stain plus kit and in a second step with Biorad coomasie blue R-250.

Photographs of embryos between stages 0 and 6 were taken with a digital camera attached to a Zeiss stereoscopic microscope. The shells of embryos at stage 7 were coated with an alloy of gold and palladium and photographed under a Phillips XL-30 scanning electron microscope at 10 x.

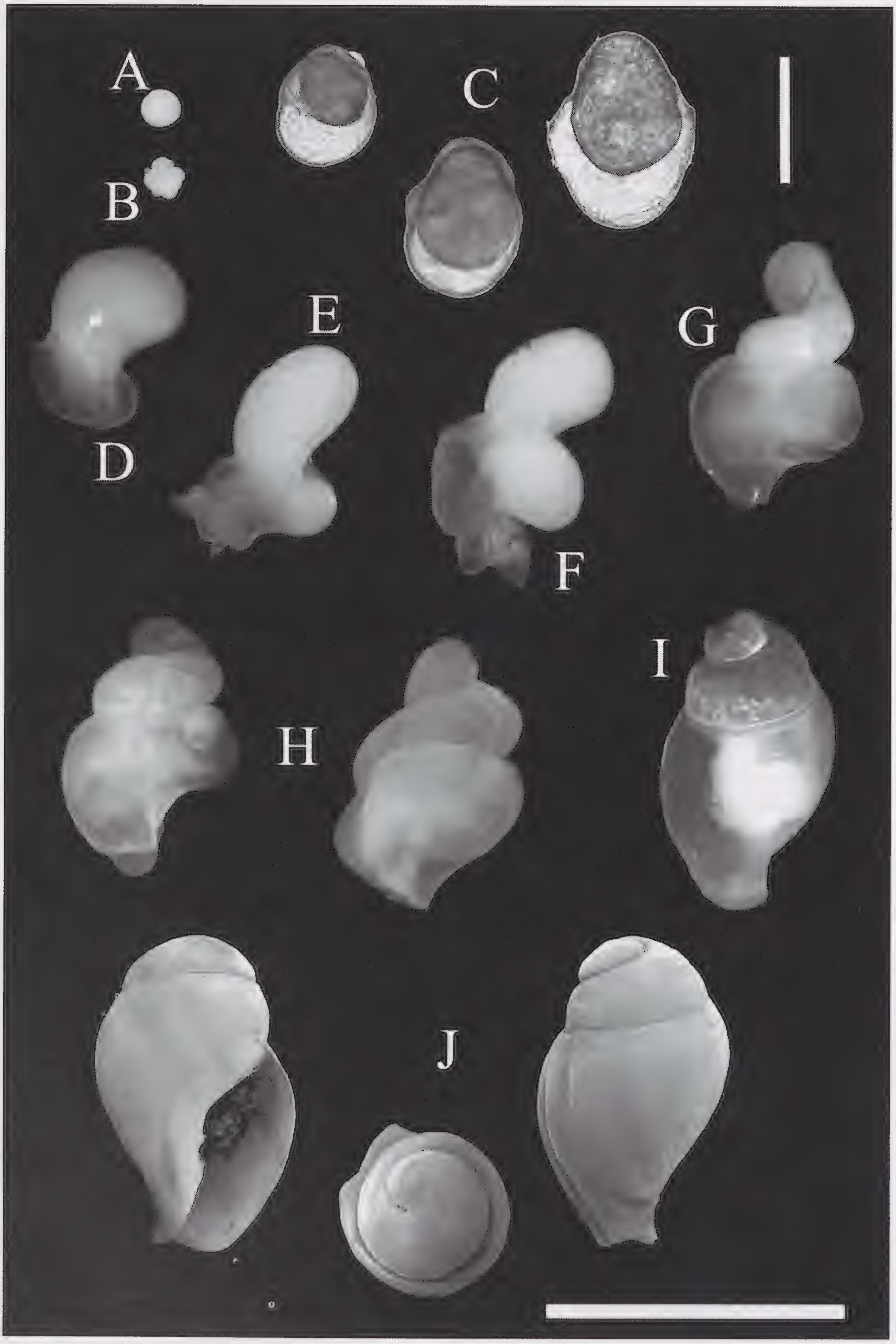

FIG. 2. Embryonic developmental stages of *Adelomelon brasiliana* according to de Mahieu et al. (1974). A: Egg; B: Blastomeres before blastulation; C: Different stages after gastrulation, stage 0; D: Stage 1, Modified veliger; E: Stage 2; F: Stage 3; G: Stage 4; H: Lateral and dorsal views of stage 5; I: Stage 6; J: Apertural, apical and dorsal view of stage 7. Vertical scale = 600 µm, horizontal scale = 1 cm.

RESULTS

Embryonic Development

The unsegmented eggs of *Adelomelon brasiliana* had a small amount of yolk (Fig. 2A), and had a mean diameter of 180 ± 8 µm. They are contained in egg capsules already released to the environment, thus indicating that the entire embryonic development occurred outside the oviduct. Subsequently, embryos undergo a slightly unequal spiral cleavage, in which macromeres do not duplicate the volume of micromers (Fig. 2 B). Development continues until the formation of gastrula. Embryo torsion starts immediately after gastrulation and results in a veliger larva with low movement and a small ciliated velum directing waterflow towards the embryo's mouth. All this developmental process was named stage 0 by de Mahieu et al. (1974) (Fig. 2C). At stage 1 (Fig. 2D), torsion is already completed and retraction of the velum starts. In addition, heart and gills become visible. At stage 2 (Fig. 2E), the second whorl is fully developed, the portion forming the rudiment of the digestive gland, gonads and some other glands can be clearly observed, and the head and foot start to develop. Some of the embryos can be seen crawling on the inner wall of the egg capsule. The rudiment of a translucent layer covering the whole embryo indicates the beginning of shell formation. At stage 3 (Fig. 2F), the embryo keeps growing, the shell is conspicuous, the third whorl starts to develop, and all embryos crawl on the inner wall of the egg capsule. At stage 4 (Fig. 2G), the third whorl is completed and body growth continues. At stage 5 (Fig. 2H), there is a compression of

TABLE 1: Embryonic developmental time, embryo sizes and number of embryos per egg capsule of *Adelomelon brasiliana* at different developmental stages.

Stage of Development	Time* (in days)	Total length (in mm)	Number of Embryos** (per egg capsule)
0	0	0.180 - 3.500	17.4 ± 4
1	5.5 ± 1.7	3.709 ± 1	15 ± 4.8
2	8.4 ± 2	4.990 ± 1.098	14.8 ± 4.2
3	11.7 ± 2.7	6.272 ± 0.559	14.5 ± 2.6
4	15.6 ± 3.6	6.946 ± 0.749	15.5 ± 3.6
5	21.5 ± 4	8.200 ± 0.437	14.1 ± 3.6
6	31.8 ± 3.6	8.571 ± 0.697	13.9 ± 3.1
7	41.2 ± 4.4	9.704 ± 0.322	15 ± 3.4
8	56.8 ± 4.3	+ 10	

* N (total) = 60
** Main effects ANOVA ($p = 0.231$, DF = 21, N = 60)

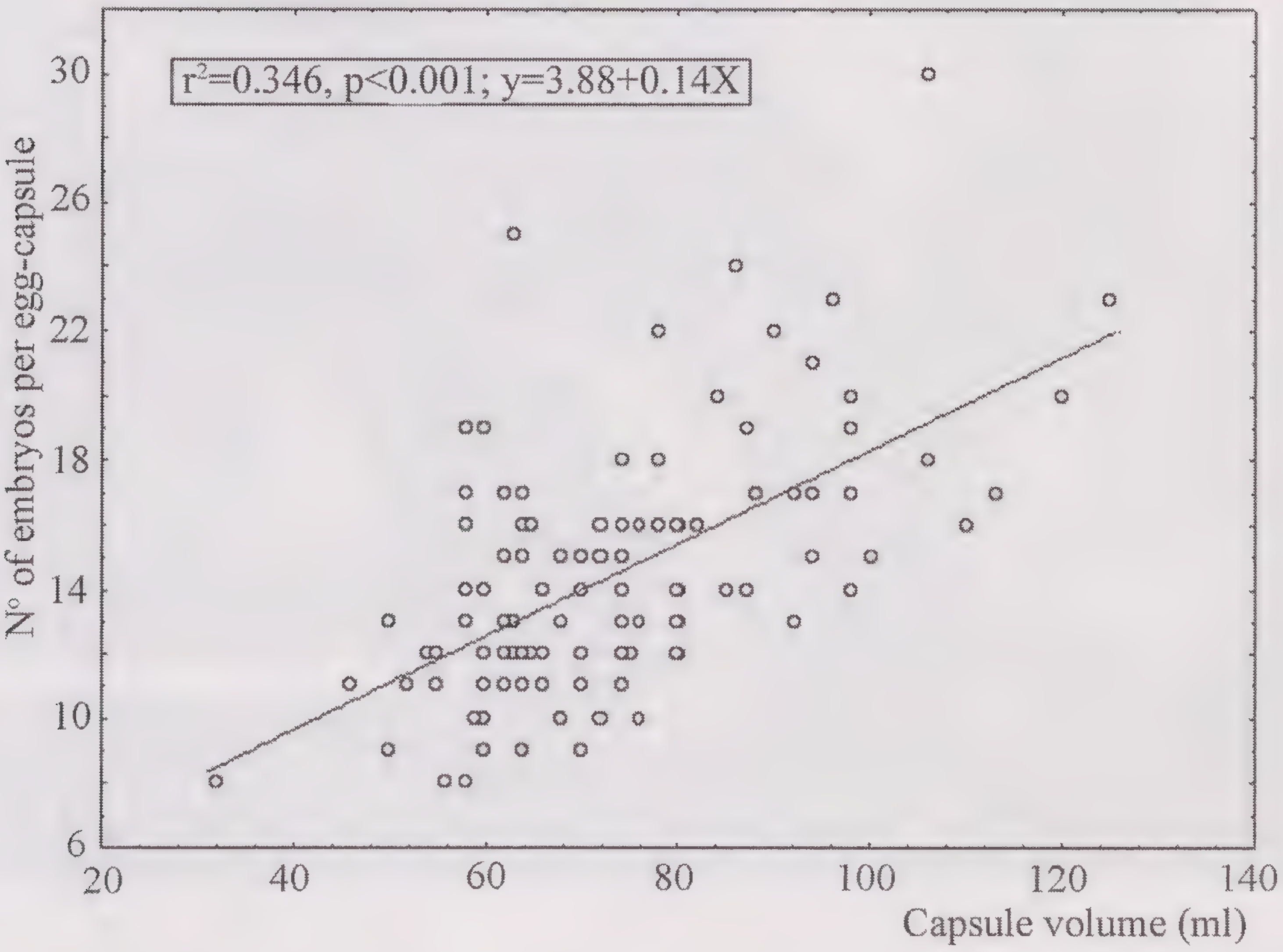

FIG. 3. Distribution of the number of *Adelomelon brasiliana* embryos in relation to egg capsule volume.

early whorls, and shell starts the calcification process. At stage 6 (Fig. 2I), the sutures of all whorls are formed, and calcification increases. These features become more evident at stage 7 (Fig. 2J), when calcification is completed and shell grows to a mean length of 1 ± 0.2 cm until hatching. In this study, hatching is included in stage 8. Embryo sizes at each developmental stage are shown in Table 1.

The number of embryos did not seem to differ among egg capsules at different developmental stages (p = 0.231; Table 1). However, there was a linear relationship between the number of embryos and egg capsule volume ($R^2 = 0.3466$, p = 0.000; Fig. 3).

Abnormal embryos were recorded (Table 2) in egg capsules at stages 6 and 7 (n = 167). The 20.36% of the egg capsules had 1 to 3 embryos smaller (38 ± 4.5%) than the expected for their developmental stages. These capsules also contained normal embryos. Teratological embryos were also found in 5.98% (n = 10) of the egg capsules. Each egg capsule contained a unique pattern of teratological embryos (Fig. 4). Teratological and normal embryos were found in eight egg capsules, while the other two only contained deformed embryos.

TABLE 2: Percentage of *Adelomelon brasiliana* egg capsules containing different numbers of teratological and underdeveloped embryos, total N = 167.

Number of Embryos	Number of teratological embryos	Number of underdeveloped embryos
1	6	17
2	2	12
3	1	5
4	1	-
Cumulative egg capsules	10	34

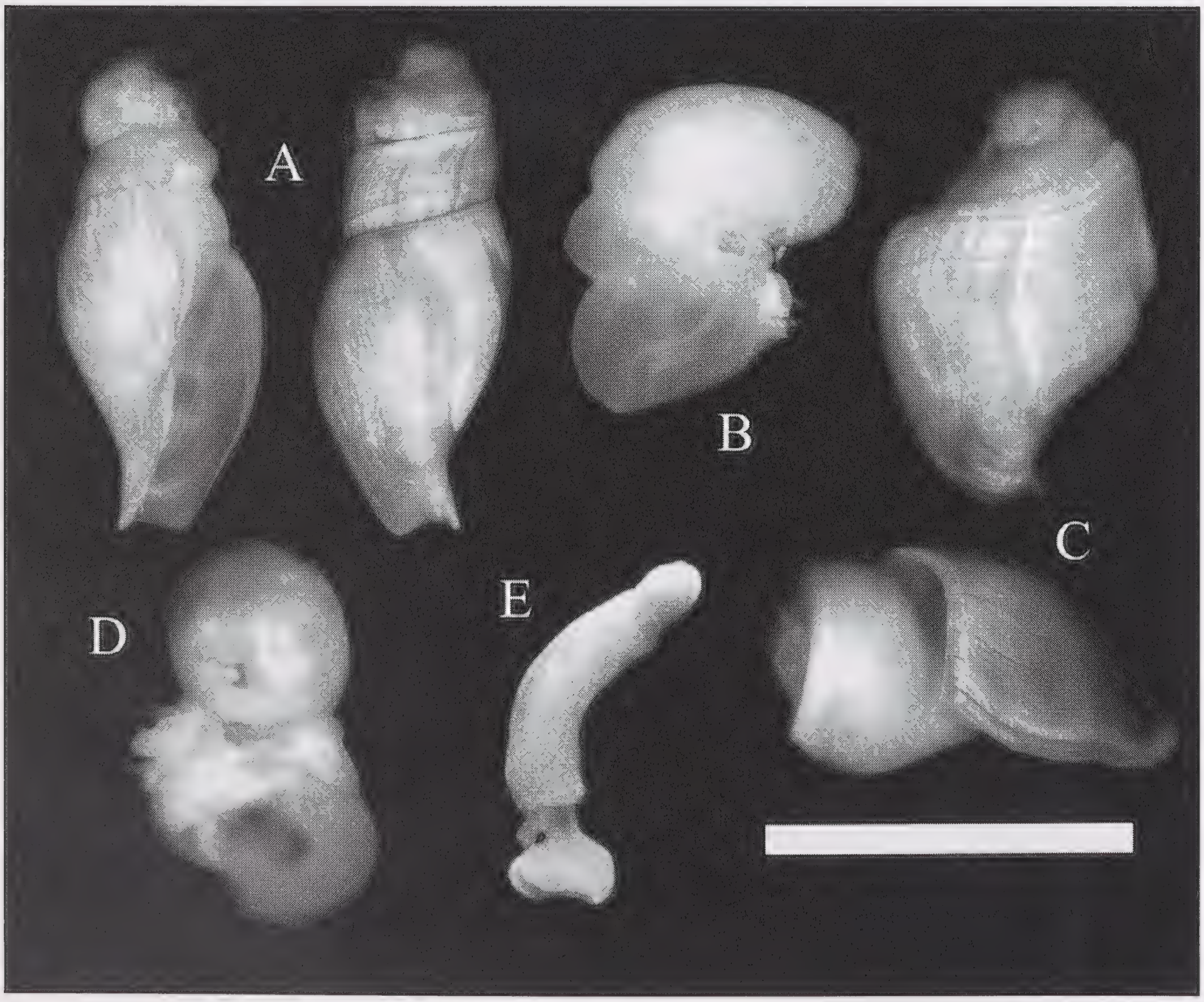

FIG. 4. Teratological embryos at stage 7 of *Adelomelon brasiliana*. A: Apertural and dorsal view of an embryo with enlarged whorls; B: Lateral view of a decalcificated embryo; C: Dorsal and lateral view of an embryo with an overlapped and abnormal growth of the whorls; D: Decalcificated embryo with abnormal growth; E: Decalcificated embryo without whorl formation. Scale bar = 1 cm.

Developmental Time

The developmental time obtained in aquaria was 56.8 ± 4.3 days, and the cumulative developmental time for each stage is detailed in Table 1.

Table 3 shows the total number of captures and the percentages of captures of egg capsules at each developmental stage. The intracapsular embryonic developmental time for each of the three study years could be estimated from this table. During the first study year, capsules in stage 0 were detected on October 28, 2000, and the first hatching egg capsules on January 24, 2001. These data indicate a maximum intracapsular developmental time of 87 days. The same analysis made for the second and third study years showed values of 84 and 98 days, respectively.

TABLE 3: Total captures (N) and percentage of developmental stages of *Adelomelon brasiliana* encapsulated embryos by sampling date.

		Developmental stage								
Date	N	0	1	2	3	4	5	6	7	8
04/27/2000	321	10.2	17.7	15.6	9.3	10.9	9.7	4.9	0.9	20.5
05/24/2000	163	28.2	22.1	11.7	8.6	8.6	4.3	1.2	1.8	13.5
06/23/2000	32	-	3.1	-	3.1	21.9	12.5	6.3	9.4	43.8
07/26/2000	49	-	-	-	-	-	-	2	-	98
08/28/2000	-	-	-	-	-	-	-	-	-	-
09/25/2000	-	-	-	-	-	-	-	-	-	-
10/28/2000	23	100	-	-	-	-	-	-	-	-
11/28/2000	69	94.2	1.5	1.4	2.9	-	-	-	-	-
12/14/2000	31	10	25.8	16.1	19.4	6.5	3.2	-	-	-
01/24/2001	52	5.8	3.8	3.8	3.8	7.7	5.8	15.4	5.8	48.1
02/20/2001	620	0.5	0.8	0.2	0.5	0.3	0.3	0.3	1.9	95.2
03/20/2001	11	9.1	36.4	-	9.1	-	-	-	9.1	36.4
04/23/2001	12	8.1	33.3	-	8.3	-	-	8.3	8.3	33.3
05//04/2001	27	59.3	-	-	-	3.7	-	3.7	-	33.3
05/24/2001	43	9.3	-	2.3	4.7	4.7	7	4.7	20.9	46.5
06/23/2001	12	-	-	-	-	-	-	-	-	100
07/26/2001	-	-	-	-	-	-	-	-	-	-
08/25/2001	-	-	-	-	-	-	-	-	-	-
09/10/2001	15	100	-	-	-	-	-	-	-	-
09/20/2001	-	-	-	-	-	-	-	-	-	-
10/20/2001	9	66.7	11.1	-	22.2	-	-	-	-	-
10/28/2001	20	100	-	-	-	-	-	-	-	-
12/04/2001	24	87.5	-	4.2	-	-	-	-	-	8.3
12/27/2001	110	32.7	30	20.9	8.2	6.4	-	-	1.8	-
01/29/2002	71	19.7	14.1	7	4.2	1.4	-	-	16.9	36.6
02/20/2002	13	69.2	30.8	-	-	-	-	-	-	-
02/26/2002	22	50	4.5	4.5	-	36.4	-	-	4.5	-
03/25/2002	62	14.5	3.2	14.5	17.7	9.7	16.1	4.8	17.7	1.6
04/20/2002	31	51.6	9.7	-	3.2	-	3.2	3.2	6.5	22.6
05/30/2002	20	60	15	-	5	-	5	-	-	15
06/31/2002	-	-	-	-	-	-	-	-	-	-
07/25/2002	-	-	-	-	-	-	-	-	-	-
08/27/2002	-	-	-	-	-	-	-	-	-	-
09/30/2002	70	100	-	-	-	-	-	-	-	-
11/08/2002	65	100	-	-	-	-	-	-	-	-
12/05/2002	222	77.5	10.8	10.8	1	-	-	-	-	-
01/08/2003	16	50	18.8	-	-	-	-	-	-	31.2
02/06/2003	116	41.38	4.3	2.6	3.4	3.4	5.2	3.4	1.7	32.8

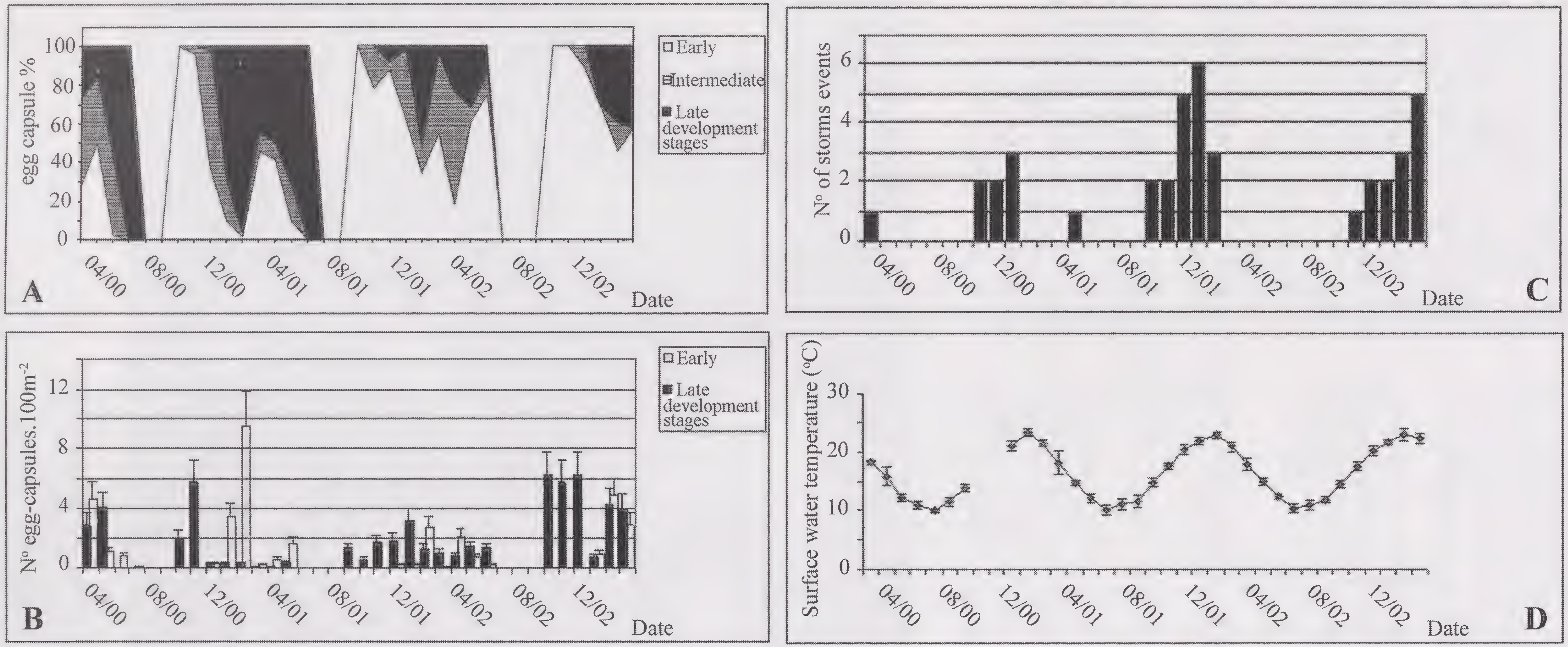

FIG. 5. A: Mean percentage distribution of encapsulated embryos stages; B: Total egg capsule densities; C: Storms surges events (San Clemente del Tuyú marigraph); D: Mean water surface temperatures (Mar del Plata marigraph).

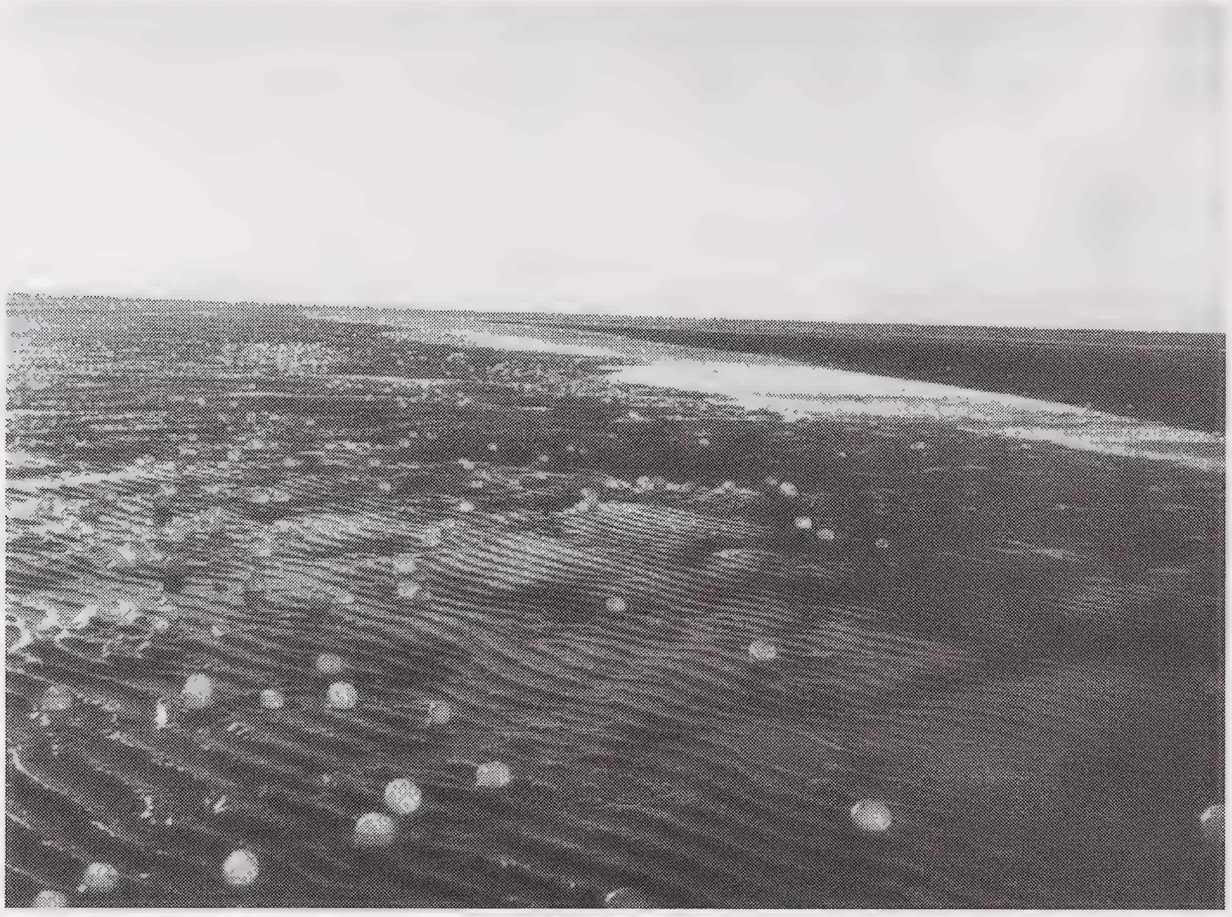

FIG. 6. Egg capsules of *Adelomelon brasiliana* stranded on a typical beach in Buenos Aires Province after storm surges events. Photograph by Ana Fazio.

Areal Abundance and Distribution Pattern over Time

Reproduction had a seasonal pattern, and egg capsules were absent during the coldest winter months. There was a correlation between mean water temperatures (Fig. 5D) and presence/absence of early developmental stages egg capsules on the sea bottom (τ =0.56, n = 34) (Figs. 5A, B).

The maximum density of egg capsules during the entire study period, regardless their developmental stage, was 30/100 m^2 in March 2000.

Egg capsules were found stranded in high densities on the emerged beach with no possibility of returning to the sea after a long period of storm surges characterized above (Fig. 6).

Recruitment was related to storm events recorded seasonally during the study period. Figures 5A and B, show a decrease in recruitment during summer 2001–2002, when surge storms were frequent and intense (Fig. 5C). In this period, recruitment was approximately 40%, whereas it was 95% for the summer 2000–2001 period.

The mean density of egg capsules collected to determine their distribution pattern was 3.55 ± 5.65 egg capsules/100 m^2, suggesting an aggregated distribution as they drift freely with littoral currents.

Biochemical Content of the Intracapsular Fluid

Protein (Fig. 7A) and carbohydrate (Fig. 7B) concentration and pH (Fig. 7C) decreased during embryo development (R^2 = 0.64, 0.47 and 0.7 respectively). The dissected capsules had a gelatinous substance on its internal face only present in the earliest developmental stages (up to stage 2). Several proteins with different molecular weights were present in the intracapsular fluid (Fig. 7D) without evident variation in composition throughout intracapsular development.

The regression between external (*ev*) and internal volume (*iv*) is significant (p = 0.000, R^2 = 0.979) and the linear equation is: $iv = 0.89\ ev$. The mean internal volume (*mv*) was 72.1 ± 14.9 ml.

The available protein per embryo in the intracapsular fluid during the embryo development (*Ped*) (Fig. 8A) was $Ped = PC_{st} \times mv / en_{st}$ where PC_{st} is the total protein per developmental stage shown in Fig. 7A and en_{st} is the media number of embryos per developmental stage (Table 1).

The available carbohydrate per embryo in the intracapsular fluid during development (*Ced*) (Fig. 8A) was: $Ced = HC_{st} \times mv / en_{st}$ where HC_{st} is the total carbohydrate per developmental stage shown in figure 7B. During the earliest developmental stages (0–2) the *Ped* and *Ced* remained constant, decreasing as the development progressed (Fig. 8).

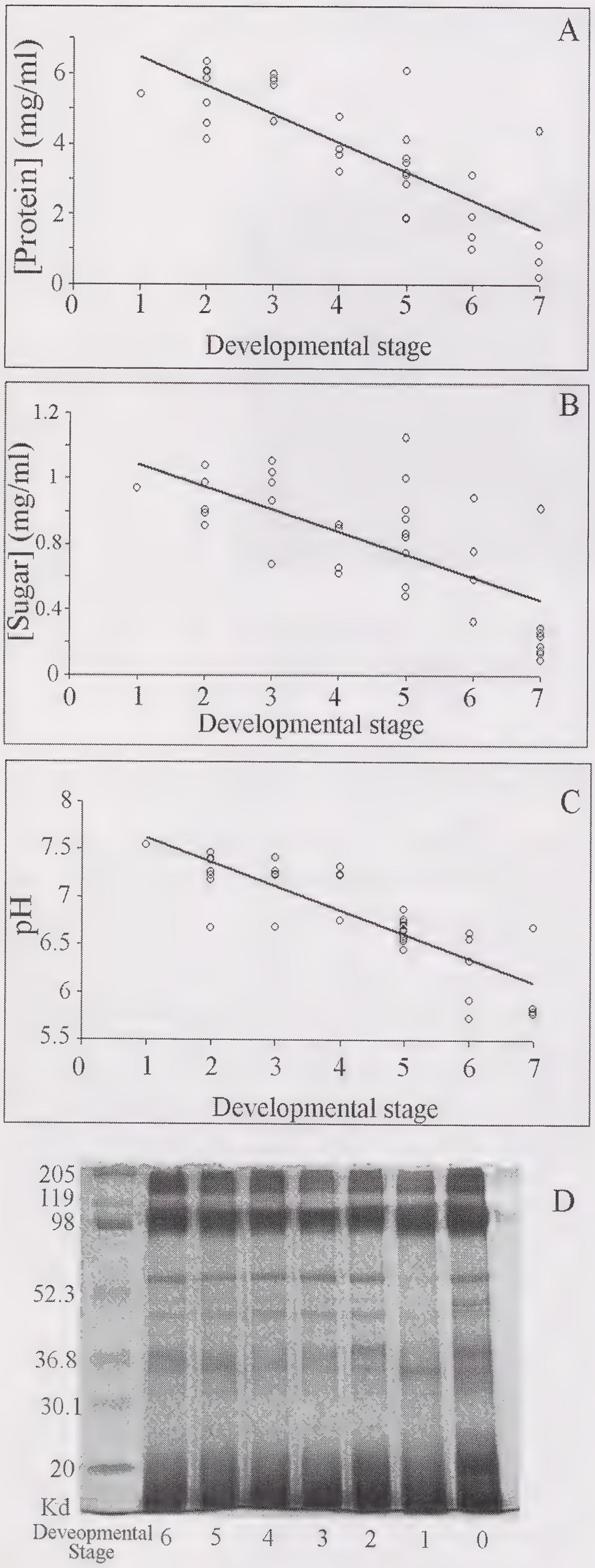

FIG. 7. Different developmental stages of *Adelomelon brasiliana*. A: Protein concentration; B: Sugar concentration; C: PH; D. SDS-page.

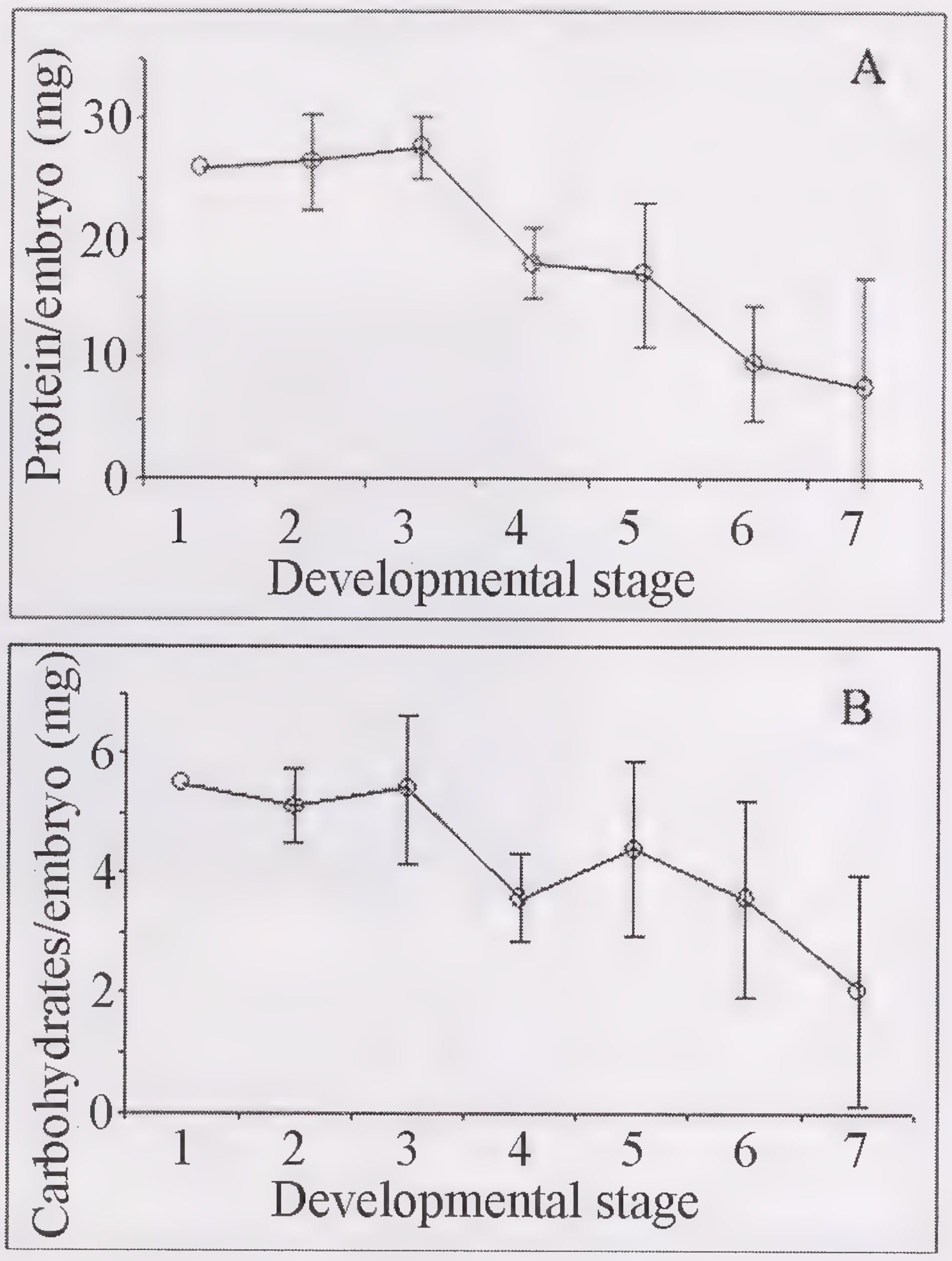

FIG. 8. Available total proteins (A) and carbohydrates (B) per embryo in the intracapsular fluid at the different developmental stages.

DISCUSSION

The small amount of yolk in uncleaved eggs of *Adelomelon brasiliana* may be related to their intracapsular development, since the intracapsular fluid contains the largest source of food available for embryonic development (de Mahieu et al., 1974). This makes possible the first cleavage of blastomeres without remarkable differences in size between macromeres and micromeres. The veliger stage of *Adelomelon brasiliana* has a very small ciliated velum providing little movement, in contrast to that of other volutid species. For example, the veliger intracapsular stage of *Voluta musica* possesses a large velum and considerable self-movement (Penchaszadeh & Miloslavich, 2001). This suggests that in *A. brasiliana*, the velum may exclusively have a feeding function at early developmental stages. The small velum disappears after stage 1, and embryos crawl on the inner wall of the egg capsule. Except for the veliger stage, no other remarkable differences in embryonic development are observed between *A. brasiliana* and the rest of the volutid species studied.

The difference between the numbers of embryos per capsule among developmental stages is not significant, although a tendency to decrease in number can be observed (Table 1). The presence of some underdeveloped or teratological embryos (Fig. 4, Table 2) could be a response of competition between developing embryos or a contamination effect, because high levels of tributyltin antifouling paint compounds (TBTs) were found inside *A. brasiliana* egg capsules (Goldberg et al., 2004) in the same study area. This could be related to the variability of embryos number not explained by egg capsule volume (Fig. 3).

The oviposition process of *A. brasiliana* is still unknown. The mean volume of egg- capsules recorded in this study was 80 ml (Fig. 2), corresponding to 9 cm in height and 7 cm in width approximately. The oviduct and vaginal opening of *A. brasiliana* are similar to those of other snail species with similar shell length (Novelli & Novelli, 1982). Although direct observation is needed to understand how the oviposition process is undertaken, these data suggest that the egg capsule of *A. brasiliana* is a loose structure, attaining its oval shape and ultimate hardness once laid down in contact with water.

González & Ibarra (2001) postulated that the climate in the study area is undergoing an effect known as “tropicalization”, which results in the concentration of rainfall during summer, together with the frequent co-occurrence of southeastern winds. According to the authors, the “tropicalization” effect shows an increasing intensity and frequency at least since the decade of 1960 when their study began. This phenomenon brings storm surges to the coastal area, leading to the stranding of invertebrates on the beach, as is the case for *A. brasiliana* egg capsules. A large number of these were found along the coast (Fig. 6), where they are subject to mass-mortality by sea-bird predation or desiccation (Penchaszadeh et al., 2000). The proportion of egg capsules at early and late developmental stages was similar during the summer period 2000–2001, whereas there was a larger proportion of egg capsules at early developmental stages in summer 2001–2002, thus indicating lower recruitment. Likewise, total abundance of egg capsules during the second study year was lower than in the first year. These results are related to the frequency and intensity of storm surges, since storm events were fewer and lasted for a shorter time in the first period than in the second (Fig. 5).

The experimental results indicate that the total developmental time of *A. brasiliana* was two months, in contrast to three months as reported by de Mahieu et al., (1974). This difference is possibly due to the methodology used, since these authors made four consecutive samplings over one reproductive season. Using the same analysis for data from the three reproductive seasons, I found that *Adelomelon brasiliana* took 87, 84, and 98 days, respectively, to complete the intracapsular development (Table 3). The values given above are possible overstimated by the monthly nature of samplings.

As in the present work, de Mahieu et al. (1974) analysed the nourishing substances in the intracapsular liquid. Penchaszadeh & Miloslavich (2001), based on that work, pointed out that the egg capsules of *A. brasiliana* have the highest amount of proteins per embryo in the intracapsular liquid among the volutid species and other families analyzed (i.e., Muricidae, Buccinidae, Melongenidae, and Marginellidae). Although information provided by de Mahieu et al. (1974) described considerably larger quantities of proteins than reported here, both results came to the conclusions referred above. There is a high-energy allocation for embryos developing in egg capsules drifting in sea currents and exposed to the risks mentioned above. However, the results can be explained in terms of availability and abundance of food resources in the environment, which is characterized by a high productivity due to fluvial processes of the Rio de la Plata estuary. In addition, *A. brasiliana* would have high reproductive effort without an insurance of its recruitment, probably due to the lack of competitors, been a carnivorous at a high trophic level.

The decrease in the total proteins and carbohydrates per embryo after stage 3 shown in Fig. 8 could be a result of a change in the food source for the embryos. During the earliest stages of development, the gelatinous layer found in the inner wall could be maintained by the protein and carbohydrates concentration of the intracapsular fluid media.

After stage 2, when the embryos change from "swimming" in the intracapsular fluid media to crawling on the inner face of the egg capsule, this capsule itself could become a source of food for the embryos that crawl and probably feed on it.

The descending intracapsular pH during embryo development was also detected by de Mahieu et al. (1974). In the present work, I found greater differences between intracapsular pH of earliest and latest developmental stages. Despite the differences recorded, we both agree that the acidification of the internal media of the egg capsule occurs. The egg capsule wall permeability is limited, since it maintains an ionic internal media different from the surrounding environment. The pH could be also limiting the time that the embryos could survive in the sealed egg capsule at values lower than 6 in latest developmental stages.

Free egg capsules in *A. brasiliana* could be interpreted as a strategy for instantaneous dispersal. Other volutid species such as *Zidona dufresnei*, *Adelomelon beckii* or *Adelomelon ancilla* that have a different reproductive mode by attaching their egg capsules to the substratum (Penchaszadeh et al., 1999; Ayçaguer, 2002), show a similar or wider geographic distribution than that of *A. brasiliana*. The free egg capsules of *A. brasiliana* may represent an adaptation to the environment, since this species inhabits sandy bottoms with almost no suitable substratum for egg capsule attachment.

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SYSTEMATIC REVISION AND CLADISTIC ANALYSIS OF *EPIPHRAGMOPHORA* DOERING FROM ARGENTINA AND SOUTHERN BOLIVIA (GASTROPODA: STYLOMMATOPHORA: XANTHONYCHIDAE)

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ABSTRACT

As a first step towards a comprehensive revision of the South American genus *Epiphragmophora* Doering, 1874, taxa described from Argentina and Bolivia, inhabitants of the rainforest Yungas (Amazonian biogeographic subregion) Monte, Pre-Puna biogeographic provinces, and Chacoan biogeographic subregion are studied. Special attention has been paid to the morphology of the terminal genitalia with respect to its relevance for systematics. The revision is based on the examination of nearly all type material, plus extensive field work and examination of additional material deposited in several museums. Shell, pallial system and genitalia of each species have been described and illustrated, some of them for the first time. Detailed description on the structure and connections of the dart sac apparatus and associated mucous glands in the terminal genitalia in each species is provided. A total of 27 species, including three new taxa, are described from the region. From the total number of species, the anatomy of seven species is described for the first time. Six unambiguous synapomorphies support the monophyly of the genus: body whorl surface malleated with diagonal ribs (character 1 [2]), umbilicus overlapping but not fused to body whorl (character 2 [1]), peristome thick, widely reflexed (character 4[1]), mucous glands unequal in size and shape (character 15 [1]), insertion of mucous glands ducts in middle portion of dart sac (character 17 [1]), and penial retractor muscle inserting in medial epiphallus (character 23 [1]). Most of the synapomorphies of the generated phylogenetic hypothesis are characters from the genitalia, and shell characters proved to be less informative. The short duct of the bursa copulatrix, a character that traditionally had been used to define the genus is characteristic only of a small group of species. The distribution of the Argentinean species studied is illustrated on maps according to the biogeographic areas. An area cladogram based on the cladistic analysis is presented. According to this hypothesis, the species inhabitants of the Yungas biogeographic province are basal groups of the remaining species inhabitants of the Chacoan, Monte, and Pre-Puna regions. The Monte and Pre-Puna species conform a monophyletic clade. The biogeographic relationships are in agreement to former studies on Latin-American entomofauna (Morrone, 2006).

Key words: Helicoidea, Xanthonychidae, *Epiphragmophora*, South America, cladistics, taxonomy, dart sac complex, biogeography.

INTRODUCTION

The genus *Epiphragmophora* Doering, 1874, is a component of the land snail family Xanthonychidae and inhabits exclusively in South America. *Epiphragmophora* is distributed in Perú, Bolivia, Paraguay and Argentina. There are also isolated occurrences in southern Colombia (type locality of *E. pilsbry* Haas, 1934) and southern Brazil (*E. bernardius* Pilsbry & Ihering, 1900). This genus is known to be a typical component of the Andean fauna, although in Argentina and Bolivia it inhabits elevated cloud rainforest areas (Yungas biogeographic subregion) and also flatlands areas of Chacoan biogeographic subregion (Morrone, 2002, 2006).

The species included in *Epiphragmophora* Doering have been catalogued by Richardson (1982) and grouped into five subgenera

(*Epiphragmophora* Doering, 1874; *Angrandiella* Ancey, 1886; *Doeringiana* Ihering, 1929; *Karlchmidtia* Haas, 1955; and *Pilsbrya* Ancey, 1887) (Thiele, 1929–1935; Zilch, 1959–1960). The monophyly of these subgenera is not yet established or even discussed. Most of the species were located by Zilch (1959–1960) in the subgenus *Epiphragmophora s. s.,* whereas the rest of the subgenera are formed by one or two species. Most of the species of the genus are known from their shell morphology, with their anatomies unknown. The anatomical information is only available for certain species classified within the subgenera *E.* (*Epiphragmophora*) and *E.* (*Doeringia*) (Hesse, 1930; Hylton Scott, 1951, 1962, 1965; Parodiz, 1955; Fernández & Rumi, 1984b; Cuezzo, 1996, 1997, 1998).

The high conchological variability found within some of the species of *Epiphragmophora* and the convergence of certain shell characters, obscure their value for the diagnosis of the genus. Previous diagnoses of the *Epiphragmophora* (Fernández & Rumi, 1984b; Schileyko, 1979; Nordsieck, 1987) are not supported by a strict character evaluation and a system of autapomorphic characters. Only one apomorphy, the presence of a penial muscular band, was identified supporting the monophyly of *Epiphragmophora* in a cladistic analysis of Xanthonychidae (Cuezzo, 1998). In the published literature, the structure of the dart apparatus, as well as the connections between mucous glands and dart sac in the different species of the genus, are not clearly described and illustrated. Due to the lack of basic anatomical knowledge, the evolution of the dart sac complex, a relevant structure for the systematics of most Helicoidean families, can hardly be reconstructed unequivocally in *Epiphragmophora*.

The objective of this study is to provide information on the anatomy of species of *Epiphragmophora* inhabiting Argentina and part of Bolivia, many for the first time, and to review their taxonomic status. Finally, to provide a cladistic hypothesis of relationship among the species reviewed those are inhabitants of two South American biogeographic units, the Yungas province (Amazonian subregion) and the Chaco province (Chacoan subregion).

Extensive field work to collect live specimens in Peru, belonging to other biogeographic provinces, will have to be done before the approximately 20 remaining nominal species of the genus, known only by their shell descriptions, can be appropriately reviewed.

Previous Classifications and Delimitations of the Genus

Doering (1874) created *Epiphragmophora* as a group within the genus *Helix*, family Heliacea. He originally described two species within *Epiphragmophora*: *E. hieronymi* Doering and *E. cuyana* Strobel. Other species now classified within *Epiphragmophora* were originally included by Doering within the groups *Aglaja* Albers and *Eurycampta* Martens.

Pilsbry (1894) included *Epiphragmophora* in the Helicidae, tribe Belogona Euadenia, characterized by having mucous glands of typically glandular structure, in contrast to the tube-like glands of the Belogona Siphonadenia. The characters described for *Epiphragmophora* by Pilsbry were the typical for the whole family because he adopted the name *Epiphragmophora* for all the American dart-bearing helices, except *Lysinoe* (= Helmintoglyptidae). The distribution of the genus, according to him, ranged then from British Columbia, Canada, to Argentina, mainly confined to the Pacific drainage, but spreading to the Gulf slope in Central America. Pilsbry also divided the genus in South American, Central and North American forms. The South American forms included three Sections, *Epiphragmophora, Pilsbrya*, and *Angrandiella*. Later, in 1939, Pilsbry recognized the error of considering the Argentine and North American helices as congeneric and then assembled all the American dart-bearing helices in the family Helminthoglyptidae. He recognized 14 genera in eight subfamilies. *Epiphragmophora* was restricted to the South American Epiphragmophorinae and considered being an "aberrant genus by its very short spermathecal duct" (Pilsbry, 1939).

Thiele (1929–1935) classified *Epiphragmophora* in the family Fruticicolidae, subfamily Epiphragmophorinae. He maintained within this genus all the American groups, dividing it into two subgenera: *Micrarionta* and *Epiphragmophora s.s.* The diagnostic characters of *Epiphragmophora s.s.* were: "shell fairly low-spire; body whorl rounded or angulated; calcified operculum (epiphragm); dart sack long, weakly curved, two edged in the center, mucous glands unequal in size, sack-shaped, short stalked; aperture non denticulate (type species: *E.* (*E.*) *hieronymi* Doering)". According to Thiele, *E.* (*Pilsbrya*) Ancey, 1887 (type species: *E. farrisi* (Pfeiffer), and *E.* (*Doeringia*) Ihering (type species: *E. trenquelleonis*

(Grateloup), were hardly separable, but he did not provide any diagnostic character of those two sections. The section *E.* (*Angrandiella*) Ancey (type species: *E.* (*A.*) *angrandi* (Morelet)) has an aperture with an unfolding below, which projects an inward tooth-like structure.

Zilch (1959–1960) adopted Pilsbry's criteria and classified *Epiphragmophora* in Helminthoglyptidae. Fernández & Rumi (1984b) reviewed the systematics of the Argentine species classifying the genus within the family Helminthoglyptidae.

Schileyko (1991) gave Epiphragmophorinae family rank defining *Epiphragmophora* with the following character combination: "There is no accessory sac. There are two mucous glands, sac-like; one of them opens directly into the stylophore or its base, another into the vagina. There is no diverticle of the spermatheca. The duct of the spermatheca is cylindrical or with a swelled base". He included in that family only the genus *Epiphragmophora* Doering with the subgenera previously established by Zilch (1959–1960).

Nordsieck (1987) maintained the subfamily Epiphragmophorinae Hoffman 1928, into Xanthonychidae, stating that its reproductive system resembles that of the Cepoliinae: diverticulum usually missing, one dart sac, dart glands unequal, one elongate and the other compact, inserting on the dart sac or on its base. Cuezzo (1998) confirmed the close phylogenetic relationships between Cepoliinae and *Epiphragmophora* through a cladistic analysis of the Xanthonychidae, in which both taxa are sister groups, whereas *Helminthoglypta* was sister group of the previously mentioned clade.

In the present study, I follow Nordsieck's (1987) suprageneric classification.

MATERIALS AND METHODS

The species examined in the present study were classified by Zilch (1959–1960) into the subgenera *E.* (*Epiphragmophora*) and *E.* (*Doeringia*). The material on which this study is based is deposited in the following institutional collections: IBNP = Inventario Biológico Nacional del Paraguay, Asunción, Paraguay; FML = Fundación Miguel Lillo, Universidad Nacional de Tucumán, Tucumán, Argentina; FMNH = Field Museum of Natural History, Chicago, USA; MACN = Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina; MLP = Museo de Ciencias Naturales de La Plata, Buenos Aires, Argentina; NKC = Museo de Historia Natural "Noel Kempff Mercado", Santa Cruz de la Sierra, Bolivia; BMNH = The Natural History Museum, London, England; MNHN = National Museum of Natural History, Paris, France; SMF = Senckenberg Museum, Frankfurt am Main, Germany.

All dimensional parameters (shell diameter; shell height, etc.) were measured using calipers. The counting of the shell whorls follows Kerney & Cameron (1979). The terms proximal and distal refer to the position in relation to the gonad. Abbreviations used in the text: D ap: apertural shell diameter; D maj: major shell diameter; Dept.: state department; det: taxonomically identified by; D min: minimum diameter; H: shell height; H ap: shell aperture height; leg.: collected by; leg. & det.: collected and identified by; Prov.: Province. Both maximal and minimum size in each kind of measurement is reported followed by the median ($\tilde{x}$) within parenthesis. In each species treated, the terminal genitalia has been drawn in a dorsal and a ventral view to see the connections and insertion points of the mucous gland ducts and their relationship to the dart sac. Type locality is given as published by the author. In the list of material used following each species description, a collection number followed by an "*", means that specimens from the species type locality have been dissected for anatomical examination. In order to avoid repetition, species descriptions are based on characters of shell, external features of the animal, jaw, terminal genitalia, plus data on the distribution and habitat preferences. Only when any particular difference was noticed in other anatomical systems (e.g., pallial complex), or when the anatomy of the species is described for the first time, a complete description of the organ or system is added. When viewing the genitalia in dorsal view after dissecting it without changing its natural position in the animal, the penial complex is located to the right of the vagina and spermoviduct; in ventral view, it will be located to the left. When referring in the text to the right mucous gland, this will always be the gland located to the right when the genitalia is viewed dorsally in natural position. General scheme for the description of genitalia follows Cuezzo (1997). Divisions into biogeographic regions for South America used in this study follows Morrone (2002, 2006).

Cladistic Analysis

The ingroup taxa used in the analysis correspond to the species of *Epiphragmophora* taxonomically reviewed in the present study. The rest of the species were not included because alcohol-preserved material was not available; in this way, about 60% of the species described for the genus (Richardson, 1982) are represented in the present analysis. Two species, *Bradybaena similaris* (Férussac), representing the family Bradybaenidae, and *Helminthoglypta tudiculata* (Binney), the type species of one of the closest genera of *Epiphragmophora*, were selected as outgroups based on previous hypotheses of relationships (Cuezzo, 1998; Wu, 2004). Cepoliinae was not included in the analysis because of the existing problems on the determination of the homology of genitalic characters and the scarce information on the anatomy of most of these genera. A complete list of characters and character states is as follows:

1 Body whorl surface: (0) with thin growth lines; (1) with thick growth ridges; (2) malleated with diagonal ribs; (3) with axial ribs regularly distributed; (4) with pustules to wrinkles.
2 Umbilicus: (0) fused; (1) with basal lip of peristome overlapped but not fused to body whorl; (2) perforate, perspective wide not overlapped by peristomal lip; (3) perforate, perspective narrow, slightly overlapped.
3 Shape of the aperture: (0) subcircular; (1) oval-horizontal; (2) subquadrangular; (3) oval-vertical.
4 Peristome: (0) thin, expanded slightly reflexed; (1) thick, wide strongly reflexed; (2) thin, highly expanded.
5 Spire: (0) high, conic; (1) shallow; conic; (2) strongly depressed.
6 Basal callus in basal peristome: (0) absent; (1) present.
7 Number of peripheral bands: (0) absent; (1) one, well-defined; (2) two or three even, continuous; (3) more than three, usually thick and interrupted.
8 Body whorl periphery: (0) convex; (1) equatorially subcarinated; (2) supra-equatorially subcarinate.
9 Aperture respect to body whorl: (0) not descending; (1) descending.
10* Diameter: (0) less than 20 mm; (1) between 21 and 29 mm; (2) more than 30 mm.
11 Number of mucous glands: (0) multiple; (1) two.
12 Shape of dart sac: (0) long, finger-like usually with constriction; (1) short cylindrical no constriction; (2) roundish.
13 Dart sac insertion: (0) in vagina; (1) in atrium; (2) in atrial sac.
14 Dart sac papillae: (0) absent; (1) present.
15 Mucous glands: (0) equal in shape or size; (1) unequal in shape or size;
16 Relation between ducts of both mucous glands: (0) separated; (1) distally fused or contiguous.
17 Point of insertion of mucous glands ducts: (0) in distal portion of dart sac; (1) in middle portion of dart sac.
18 Position of left mucous gland duct: (0) distal respect to the body of the gland; (1) ecuatorial respect to the body of the gland.
19 Presence of a sac-like mucous glands in terminal genitalia: (0) absent; (1) present.
20 Right mucous gland: (0) not fused with atrium wall; (1) distally fused with atrium wall.
21* Penis length respect to epiphallus (0) half epiphallus length; (1) as long as epiphallus; (1) longer than epiphallus length.
22 Penial papillae (= verge): (0) absent; (1) present.
23* Penial retractor muscle: (0) inserts in distal epiphallus; (1) inserts in medial zone of epiphallus; (2) inserts in proximal epiphallus.
24* Duct of bursa copulatrix: (0) extremely short, not longer than sac; (2) medium; (3) long.
25 Bursa copulatrix's diverticulum: (0) absent; (1) present.
26* Length of vagina: (0) short; (1) medium to long; (2) extremely long.
27 Atrium: (0) short, not expanded; (1) medium to long, not expanded; (2) long, prolonged into an atrial sac.
28 Flagellum: (0) absent; (1) triangular, thick, curved, short; (2) finger-like short to medium; (3) thin, long.
29 Penial muscular band: (0) absent; (1) present.
30 Penial sheath (= penial-tucica): (0) simple; (1) double or multilayer.
31 Habitat: (0) in association to rocks; (1) in association with tree logs.
32 Vas deferens: (0) passing by the constriction of dart sac; (1) not.
33 Epiphallus proximal portion: (0) Not widen at point of entrance of vas deferens; (1)

widen at point of entrance of vas deferens.

34 Penial retractor: (0) not forming a loop around vas deferens before inserting in epiphallus; (1) forming a loop.

35 Secondary ureter: (0) open; (1) closed.

A total of 35 morphological and ecological characters from shell morphology (10), genital system (23), pallial system (1), and one corresponding to habitat preferences were selected from the 29 species examined. These selected characters for the taxa examined were coded in a matrix (Appendix). From the total number of characters, 18 were coded as multistate (characters 10, 21, 23, 24 and 26 are additive) and the remaining 17 as binary. The symbol "?", in the matrix means "character state unknown". For the cladistic analysis of the character matrix, the computer program PeeWee 2.9 (Goloboff, 1993) was used. PeeWee is a program for parsimony analysis under implied weights. The weighting method implemented in PeeWee is explained in detail by Goloboff (1993, 1995). The ingroup taxa were rooted in Bradybaenidae, representing the closest Helicoidean family of the Xanthonychidae (Cuezzo, 1998; Wu, 2004). Alternatively, *Helminthoglypta tudiculata* was also used to root the ingroup. With PeeWee parsimony program the characters are weighted in inverse relation to the amount of homoplasy (extra steps) they show on each examined tree. Trees with highest total fit or "optimal trees", are retained in the search. The command "mult*100", was used through which the order of the taxa were randomized, a Wagner tree was created and submitted to branch-swapping (TBR), and 1,000 trees each time were stored in memory. This process was repeated one hundred times. Bremer support, which evaluates the support for clades, was calculated with the command "bs", implemented in PeeWee (Command sequence: "suboptimal 300; hold 1000; max*; bsupport"). To facilitate character evaluation and tree illustration, the computer program WinClada 1.00.08 (Nixon, 2002) was used. Two different searches were done with the created matrix. In the first, all species included in the matrix were treated and analyzed as terminals. In a second step, species known only by shell characters were eliminated from the matrix and a new search for optimal trees was conducted. Species known only by shell characters are indicated with underlined letters in the matrix (Appendix).

RESULTS

Epiphragmophora Doering 1874

Helix group *Epiphragmophora* Doering, 1874

Helix group *Aglaja* Albers [partim] – Doering, 1874: 445.

Helix group *Eurycampta* Martens [partim] – Doering, 1874: 448.

Epiphragmophora – Pilsbry, 1894: 193; Parodiz, 1957: 29; Zilch, 1959–1960: 655; Fernández, 1973: 157; Fernández & Rumi, 1984b: 231.

Type Species: Epiphragmophora hieronymi Doering, 1874, by original designation.

Diagnosis: Shell with one to three dark distinctly peripheral bands around body whorl; protoconch smooth; aperture with medium to thick calcareous epiphragm; aperture without tooth in most species, only one species known to have a single basal tooth; terminal genitalia with dart sac complex (= stimulatory complex) having a single dart sac and two unequal mucous glands, some species with one sac-like mucous gland enveloping basal vagina and dart sac complex, in others both mucous glands cylindrical, oval to club-shaped; efferent ducts of mucous glands fused or separated, inserting in medial zone of dart sac; single dart sac cylindrical, usually with median constriction bellow with mucous glands insert; dart sac inserting in vagina before joining with penial complex or in atrium level with vagina and penial complex; single internal, long dart gland usually present in distal dart sac; bursa copulatrix without diverticulum, duct with variable lengths; penial papilla (= verge) generally short present in some species; penial muscular band present in some species; epiphallus generally shorter than penis, externally not well differentiated from it; penial retractor muscle inserting in middle zone of epiphallus; penial sheath usually present, sometimes double or multiple layered.

Distribution: The distribution of *Epiphragmophora* in Argentina ranges from northern Jujuy to central Cordoba and San Luis provinces. The western part of the country is the species richest area. The genus inhabits a variety of ecoregions from rainforest of the Yungas to the xerophilic environments of Monte, Pre-Puna and Chacoan regions. Vertical distribution ranges from lowlands (200–400 m above sea level) to highlands of about 3,500 m above sea level. In Bolivia, *Epiphragmophora* is distributed from the Cordillera Oriental and Sierra Subandina in the western part of the country to the Serranias Chiquitanas in southeastern Bolivia. They inhabit very different environments, from Yungas (Tucumano-Boliviano forests) to xerophilic ecoregions such as Chaco and Chaco Serrano (Ibisch et al., 2003).

Remarks: According to previous phylogenetic hypothesis (Nordsieck, 1987; Cuezzo, 1998), *Epiphragmophora* is most closely related to Cepoliinae and *Helminthoglypta*. Zilch (1959–1960) arranged the genus into five subgenera, but their status as natural monophyletic groups has not been tested. Further comparative anatomical information among the different species is needed before the acceptance of the classification into subgenera.

Epiphragmophora argentina (Holmberg, 1909)
(Figs. 1A, 8A, B, 20)

Helix argentina Holmberg, 1909b: 91.
Epiphragmophora tucumanensis (Doering) – Hesse, 1930: 137.
Epiphragmophora argentina – Parodiz, 1957: 30.
Epiphragmophora argentina – Fernández & Rumi, 1984b: 236.

Neotype: MACN 9913, Argentina, Tucumán, Cadillal, M. Doello Jurado, 22/III/1919.

Material Examined:
Neotype: MACN 9913, Argentina, Tucumán, Cadillal, M. Doello Jurado, 22/III/1919
Dissected Material: Argentina, Tucumán Prov.: FML 14415 A, Tafí Viejo Dept., Raco, 1/VII/1996, between *Bromelia* leaves, E. Dominguez leg. *FML 14406, Tafí Viejo Dept., El Cadillal, on road to Aguas Chiquitas, 26°36’48"S, 65°11’11"W, 560 m, 13/VIII/1990, M. G. Cuezzo leg. & det. FML 114 A, Yerba Buena Dept., San Javier, II/1990, M. G. Cuezzo leg. FML 14408 A, Burruyacu Dept., Rio Nio, 1/XII/1996, M. G. Cuezzo leg. & det. FML 14411 A, Tafi Viejo Dept., El Cadillal, Aguas Chiquitas, 7/III/1990, M. G. Cuezzo leg. FML 14412 A, Yerba Buena Dept., in a garden of “Las Lomitas”, close to Horco Molle, 8/V/1995, Molineri, C. leg. FML 14409 A, Trancas Dept., Tapia, road branching from route 341 at km 13, between Raco and route 9, 6/IV/2003, M. G. Cuezzo leg. & det. FML 14416 A, Yerba Buena Dept., San Javier, University of Tucumán Ville, 9/III/2001, M. G. Cuezzo leg. FML 14417, Tafi Viejo Dept., San Javier, 7 km N from street 3, 1/I/1999, M. G. Cuezzo leg. & det.
Dry Material: Argentina, Tucumán Prov.: MACN 9913-1, Tapia Dept. FML 543, Yerba Buena Dept., San Javier, 600–650 m, 3/III/1962, W. Weyrauch leg. & det. FML 753, Yerba Buena Dept., San Javier mountains close to Tucumán city, 600–650 m, 1967, W. Weyrauch leg. & det. FML 75, El Saladillo, 8/IV/1947, Parodiz, J. det. FML 11049, Quebrada de La Hoyada, 1/IX/1936, Dionisio Ollea leg., W. Weyrauch det. FML 14284, Aconquija, W of city of Tucumán, 650–700 m, 1/VI/1970, leg. & det. by W. Weyrauch. FML 1392, Yerba Buena Dept., Horco Molle, 700 m, 4/II/1968, leg. & det. by W. Weyrauch. FML 765, Burruyacú Dept., Rio Nio, 900 m, 25/V/1967, leg. & det. by W. Weyrauch. FML 747, Trancas Dept., near Tapia, on road from Tucumán to Tapia, 700 m, 18/VI/1967, leg. & det. by W. Weyrauch. FML 14276, J. B. Alberdi Dept., Escaba, 1/VII/1998, F. Drahg leg. FML 14402, Trancas Dept., Ticucho, 15 km from El Cadillal and 19 km from route 9, 1/XII/1998, M. Maya leg. FML 14400, Tafi Viejo Dept., El Cadillal, Aguas Chiquitas, 30/VIII/1986, M. G. Cuezzo leg. FML 62, Tafí Viejo Dept., El Duraznito, 6/V/1947, W. Weyrauch det. FML 11052, Tafi Viejo Dept., San Javier, La Cascada, 1,400 m, 6/I/1964, Meyer, T. leg., Weyrauch det. FML 1449, Tafí Viejo Dept., El Cadillal, 600 m, 17/VIII/1968. leg. & det. W. Weyrauch. Argentina, Salta Prov.: FML 85, El Morenello, Rosario de La Frontera, 1/X/1936, R. Schreiter leg.

Description:
External Features: Animal light brown to pale yellow; homogeneous color over cephalopedal region; two lines of pustules separated by groove running dorsally from mantle collar to cephalic region ending between ommatophores; dorsal groove in pedal region well marked.
Shell (Fig. 1A): Dextral, helicoidal, globose, solid, elevated conical spire; small to medium size, 4½ to 5 convex whorls; color variable from light yellow to dark brown; single thick equatorial, dark brown even band in body whorl periphery; protoconch smooth; teleoconch with growth lines in spire and

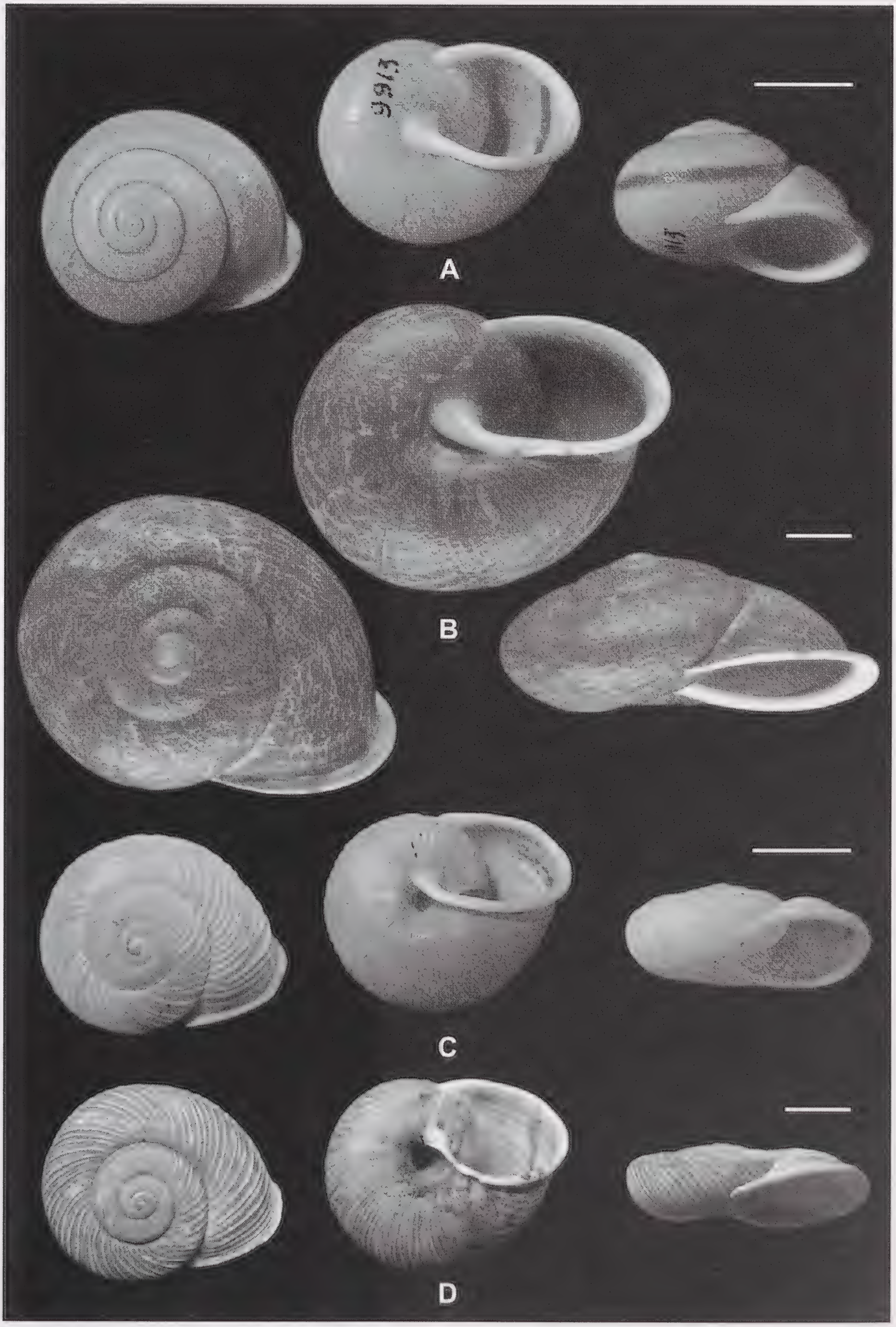


FIG. 1. Shells in dorsal, ventral and lateral view. A: *Epiphragmophora argentina* (MACN 913, neotype). Scale bar = 15 mm; B: *Epiphragmophora audouini* (MNHN, syntype). Scale bar = 10 mm; C: *Epiphragmophora birabeni* (MACN 23034, holotype). Scale bar = 15 mm; D: *Epiphragmophora costellata* (MLP 4291, holotype). Scale bar = 10 mm.

surface strongly malleated in body whorl; suture deep impressed; aperture subcircular; peristome whitish, thick, reflexed; body whorl distinctly descending towards aperture; generally imperforated, some specimens with umbilicus totally overlapped by basal lip but not fused with parietal wall.

Measurements: Neotype: D maj: 27.6 mm; D min: 23 mm; H: 18.4 mm.

Other Material (n = 20): D maj: 31.4–24.1 mm (28.5 mm); D min: 27.5–21.6 mm (24.4 mm); H: 19.5–15.3 mm (17.4 mm); D ap: 21.0–16.1 mm (18.5 mm); H ap: 17.0–13.8 mm (14.8 mm).

Jaw: Corneous, thin, orange-translucent; three vertical wide ribs on central jaw with two other wider plaques on each side of central ones; vertical plaques projecting at both jaw edges.

Pallial Complex: Kidney triangular not exceeding half pulmonary roof length; main pulmonary vein thick, splitting into two secondary branches before reaching mantle collar; pulmonary roof between main pulmonary vein and rectum furrowed by minor veins; some specimens with lung mottled with dark spots.

Genitalia (Fig. 8A, B): Terminal genitalia with dart sac complex having two mucous glands symmetrically located in either side of dart sac, both elongated to cylindrical in shape, with thin ducts inserting independently in middle zone of dart sac; duct of right mucous gland (dorsal view) distally located with respect to body of gland, while duct of left mucous gland equatorial with respect to body of gland; dart sac muscular, cylindrical, even in diameter, inserting in medial portion of vagina; internal wall of dart sac with uniform longitudinal bands, dart papillae thick and short in distal dart sac; bursa copulatrix elongate-oval, extending towards middle zone of spermoviduct; duct of bursa copulatrix long, convoluted, running parallel to distal portion of spermoviduct; penial complex with long, thin, convoluted flagellum, transition between epiphallus and penis not evident externally but internally through different wall sculpture; proximal portion of epiphallus thickening at point of insertion of vas deferens; internally wall sculpture of epiphallus consisting of straight longitudinal bands; epiphallus thin, slender, as long as penis; wall sculpture of proximal penis with thin zigzag longitudinal bands, distal penis with closer columns in a zigzag pattern; penial papillae absent; penial sheath simple, muscular, enveloping penis in its complete length, distally fused with atrium; penial muscular band long, located under penis sheath; penial retractor muscle thick, inserting in middle portion of epiphallus; penis and vagina entering side by side in atrium, distally to dart sac complex; atrium long.

Habitat: *Epiphragmophora argentina* is found under or inside tree trunks on the ground in both xerophilic and humid forests. Rarely found adhering to rocks.

Distribution (Fig. 20): *Epiphragmophora argentina* is distributed mainly in Tucumán Province, northwestern Argentina, where is very abundant. There are some other scarce records in Catamarca and Salta provinces. This species is found in subtropical rainforest (Yungas biogeographic province), xerophilic forests and also in the Chacoan biogeographic subregion (Morrone, 2006).

Remarks: *Epiphragmophora argentina* has often been confused with *E. tucumanensis* (Doering) probably because they are partly simpatric in their distribution. Hesse (1930) published an anatomical study on *E. tucumanensis* based on an erroneously identified material of *E. argentina.* The holotype of Holmberg from the locality of Tapia in Tucumán was lost, and J. J. Parodiz selected a neotype for *E. argentina* from El Cadillal, Tucumán (MACN 9913). This species is one of the most abundant of the genus and is easily collected during rainy days. *Epiphragmophora argentina* differs from *E. tucumanensis* in having a sealed shell umbilicus or narrower open one. *Epiphragmophora tucumanensis* is usually bigger in shell diameter than *E. argentina,* with darker shells and less globose body whorl. *Epiphragmophora argentina* has both the flagellum and epiphallus, thinner and longer than in *E. tucumanensis.* The epiphallus in *E. argentina* is as long as the penis, while in *E. tucumanensis* it is half the penis length. They also differ in the structure of the penial sheath, which is simple and thin in *E. argentina* and it is double and muscular in *E. tucumanensis.*

The original description of the present species indicates that the shell umbilicus is sealed to the wall of the body whorl; however, in the type locality of *E. argentina*, several specimens with umbilicus not fused but overlapped by peristomal lip were collected. Later examination of the anatomy confirmed those specimens as *E. argentina.*

The original locality of the type designated by Holmberg (1909b) was in Tapia, Tucumán, a region with xerophilic vegetation and dry substratum of the Chacoan biogeographical subregion. The locality of the neotype designated by Parodiz (1957) is from El Cadillal, Tucumán, a locality with subtropical rainforest in the Yungas biogeographical province. Yungas regions are typical areas inhabit by *E. argentina*, but some Chacoan localities of occurrence are also registered for this species.

Epiphragmophora audouini (d'Orbigny, 1835)
(Fig. 1B)

Helix (*Lysinoe*) *audouini* d'Orbigny, 1835: 239, pl. 24, figs. 4–6.

Material Examined:
Syntypes: MNHN, Bolivia, Yungas. BMNH, Bolivia, Yungas, Carcuata.
Type Locality: Bolivia, La Paz Dept., Yungas and Inquisivi Prov., between the villages of Chirca and Chulumani, environments of Circuata, Carcuata and Suri (for a precise locality: Breure, 1973).

Description:
Shell (Fig. 1B): Dextral, helicoidal, globose, solid, subcarinated with conical spire; large size, 4¾ convex whorls, dark brown, no peripheral band evident in the body whorl; protoconch smooth; teleoconch surface malleated with diagonal ribs on body whorl; suture deep impressed; aperture elliptical, peristome white, strongly expanded, with strong callus in parietal wall; body whorl distinctly descending towards the aperture; umbilicus narrow, almost totally overlapped by ventral columelar fold of the peristome.
Measurements: Syntype: D maj: 58.8 mm; D min: 50.6 mm; H: 31.8 mm; D ap: 36.6 mm; H ap: 27.4 mm.

Remarks: Neither live specimens nor empty shells were found by the author in the type locality in Bolivia; one possible reason was that the type locality area has been greatly modified since d'Orbigny visited. Pilsbry (1890) considered that *H. audouini* d'Orbigny has a variety, *H. a. var. oresigena* d'Orbigny. Here *E. oresigena* (d'Orbigny) is considered a species different from *E. audouini* (d'Orbigny) on the base of shell characters of the type material. Materials corresponding to other syntypes of the species deposited at NHM were examined from photographs. *Epiphragmophora audouini* differs from *E. oresigena* in having larger shell diameter (D maj = 58.8 mm; *E. oresigena* D maj = 39.9 mm), shells of *E. audouini* usually don't have a peripheral band, whereas in *E. oresigena* three pigmented peripheral bands are present. The shell aperture in *E. audouini* is elliptical in shape, whereas in *E. oresigena* the shape of the shell aperture is subquadrate. Other marked differences between these two species are in the peristome; in *Epiphragmophora audouini* the peristome is strongly expanded, whereas in *E. oresigena* the peristome is reflected.

Epiphragmophora birabeni Parodiz, 1955
(Fig. 1C)

Epiphragmophora birabeni Parodiz, 1955: 93, fig. 1.
Epiphragmophora birabeni – Parodiz, 1957: 29
Epiphragmophora birabeni – Fernández & Rumi, 1984b: 238.

Material Examined:
Holotype: MACN 23034, Argentina, Catamarca Prov., Quebrada de La Hoyada. R. Schreiter leg., 1936.

Description:
Shell (Fig. 1C): Dextral, helicoidal, globose, translucent, delicate, spire shallowly elevated, medium size, 3½ convex whorls, yellowish to golden; peripheral pigmented band in body whorl absent; protoconch smooth; sculpture of teleoconch with delicate axial whitish ribs regularly distributed, more spaced and thicken in body whorl, thin axial undulated or concentric lines between ribs giving the appearance of fingerprints; suture sinuous, well impressed; aperture transverse oval, peristome white, thick, reflexed; body whorl abruptly descending behind aperture; umbilicus deeply perforated and partially overlapped by columelar fold of peristome.
Measurements: Holotype: D maj: 25.6 mm; D min: 23.2 mm; H: 11.4 mm.

Distribution: This species is only known from the type locality.

Remarks: *Epiphragmophora birabeni* is very similar to *E. saltana* in shell morphology. Differences between the two species are: the sculpture of the inter-rib spaces, absence of peripheral pigmented band in *E. birabeni* and shell size. *Epiphragmophora saltana* is bigger in shell diameter than *E. birabeni* and posseses a dark peripheral band in the body whorl. The type locality of *E. birabeni* is La Hoyada in Catamarca, located in higher mountains at more than 3,000 m altitude corresponding to the Pre-Puna biogeographic region. It is an almost desert place with scarce vegetation. It is possible that *E. birabeni* inhabit in narrow rock crevices, although no live specimens were found. Due to this situation *E. birabeni* is still known from a single specimen (the holotype) deposited at Museo Argentino de Ciencias Naturales (MACN).

Epiphragmophora costellata Fernández & Rumi, 1974
(Figs. 1D, 20)

Epiphragmophora costellata Fernández & Rumi, 1984a: 217; 1984b: 238.

Holotype: MLP 4291, Argentina, Salta Prov., "El Rey" National Park. Obregoso & Brown leg.

Other Material Examined:
Dry Material: MLP 4291 Holotype and Paratypes; MLP 4292 Paratypes, Argentina, Salta Prov., "El Rey", National Park. MPL 4310, Argentina, Salta Prov., Cerro EL Chañar, "El Rey", National Park, Brown, A. leg., 1981.

Description:
Shell (Fig. 1D): Dextral, planorboid to discoidal, solid, spire depressed, large, 4¾ whorls, with body whorl supraequatorally slightly angled at periphery; whitish to light brown; thin, supraequatorial pale brown peripheral band evident in last two whorls; protoconch smooth; body whorl with strong axial ribs regularly separated, less marked in upper whorls, spaces between ribs with irregularly arranged granules; suture sinuous, well impressed; aperture subcircular; peristome thin, white, reflexed; body whorl slightly descending behind aperture; umbilicus wide, deeply perforated, partially overlapped by basal lip.
Measurements: Holotype: D maj: 38.0 mm; D min: 30.0 mm; H: 13.3 mm.
Paratypes (n = 7): D maj: 35.0–40.0 mm (37.5 mm); D min: 30.6–34.6 mm (32.2 mm); H: 14.3–16.8 mm (15.7 mm).

Habitat: Between and below rocks in grassland (= pastizal) on top of mountains, this environment is classified as belonging to the Yungas biogeographic province, Amazonian subregion of the Neotropical region (Morrone, 2006).

Distribution (Fig. 20): *Epiphragmophora costellata* is only known from the type locality in Cerro Maldonado and in Cerro "El Chañar", both localities from the Argentinean National Park "El Rey", Salta Province.

Remarks: *Epiphragmophora costellata* is similar to *E. birabeni* and *E. saltana* in the presence of regular axial ribs on the shell. Major differences among the three mentioned species are found in shell microsculpture among the ribs, in the spire, in general shell shape and size.

Epiphragmophora cryptomphala Ancey, 1897
(Figs. 2A, 8C, D)

Epiphragmophora cryptomphala Ancey, 1897: 10.
Epiphragmophora trigrammephora cryptomphala – Parodiz, 1957: 31.
Epiphragmophora cryptomphala – Fernández & Rumi, 1984b: 239.

Material Examined:
Dissected Material: FML 14760 A, Argentina, Salta Prov., Ledesma Dept., on road to Valle Grande, 30/I/1990, E. Dominguez leg.
Dry Material: Argentina, Salta Prov.: FML 14382 (ex 195523), La Quena, Bermejo river, 6 km S of Embarcación, 270 m, 22/IX/1968, leg. & det. by W. Weyrauch. FML, Salta, 3/I/1990. E. Dominguez, leg. FML 852, Oran Dept., 6 km S of Embarcación, close to Bermejo river, 270 m 27/II/1964, leg. & det. by W. Weyrauch. FML 323, Oran Dept., VIII/1944, leg. A. Castellanos. MLP 4299, Orán. MLP 1667, Embarcación, Hylton Scott-Biraben leg., 27/II/1945. MLP unnumbered, Manuel Elordi, Birabén, M. leg. 22/V/1947, ex collection Hylton Scott. Argentina, Jujuy Prov.: MLP 4284, Viñalito.
Argentina, Formosa Prov.: MLP unnumbered, Santa Victoria, Prosen, A. X/1948, ex col. Hylton Scott.

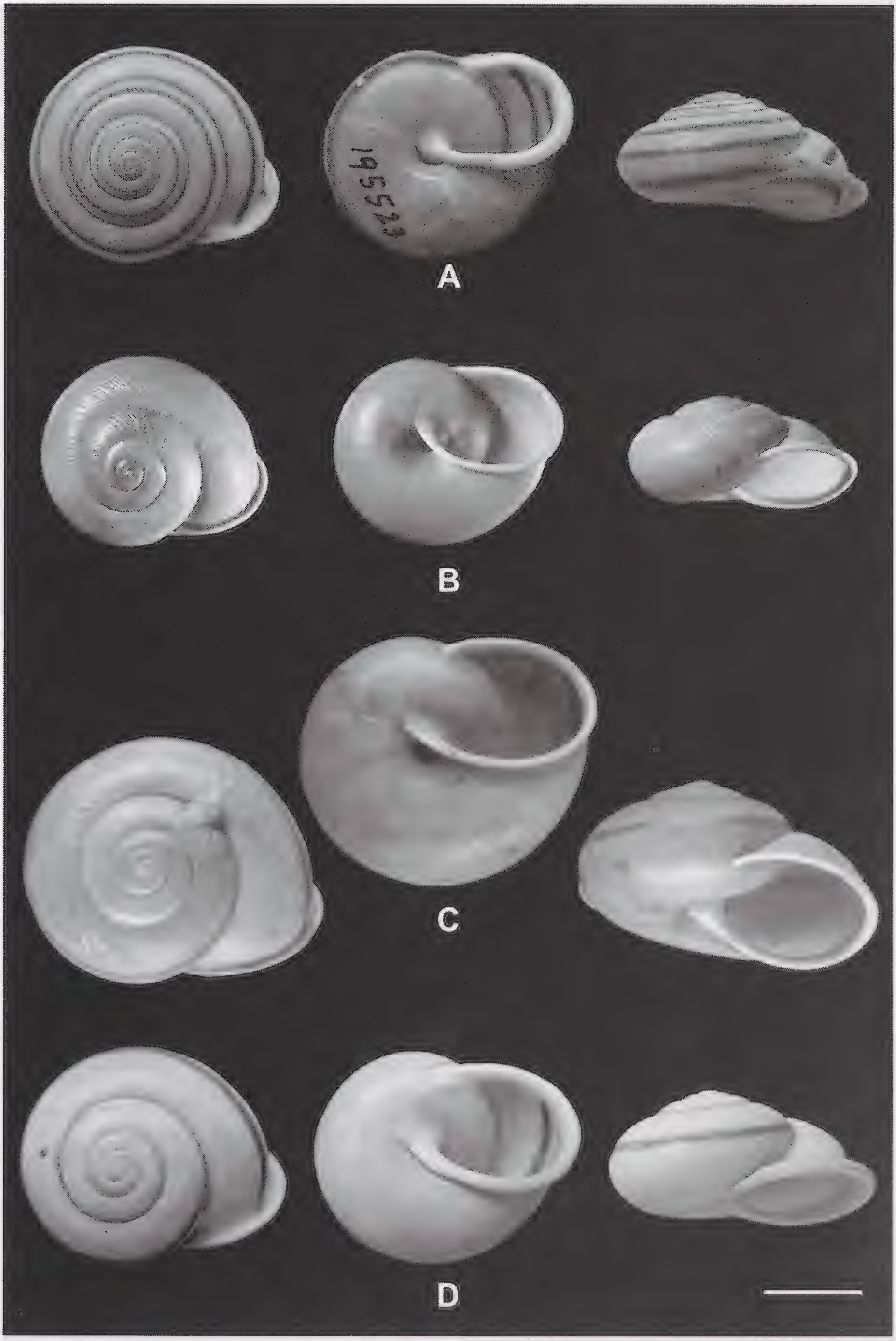


FIG. 2. Shells in dorsal, ventral and lateral view. A: *Epiphragmophora cryptomphala* (FML 14382); B: *Epiphragmophora escoipensis* (FML 14090, holotype); C: *Epiphragmophora estella* (MNHN, syntype); D: *Epiphragmophora hemiclausa* (MLP, holotype). Scale bar = 15 mm.

Description:

External Features: Body color homogeneous light gray to pale brown.

Shell (Fig. 2A): Dextral, helicoidal, subglobose, semisolid to solid, with conical spire, usually high; medium size, 4½ convex whorls; whitish, pale cream to yellowish; three peripheral thin, darker brown bands clearly visible on spire and body whorl, usually ventral band wider than others; protoconch smooth, first whorls with thin growth lines; teleoconch surface malleated with diagonal ribs on body whorl; suture deep impressed; aperture subquadrangular or subovoidal, peristome white, wide, expanded, slightly reflexed; body whorl abruptly descending towards aperture; umbilicus completely overlapped by ventral columelar fold of peristome; in some specimens peristome partially fused with umbilicus.

Measurements: Specimens from Salta (n = 15): D maj: 28.6–22.9 mm (25.9 mm); D min: 25.3–20.9 mm (23.3 mm); H: 15.3–11.9 mm (13.8 mm); D ap: 18.0–13.8 mm (16.10 mm); H ap: 13.6–10.9 mm (12.5 mm).

Jaw: Thin, translucent; three shallow central plaques with only concave border of central vertical plaque exceeding border of jaw.

Genitalia (Fig. 8C, D): Terminal genitalia with single dart sac and two mucous glands, left one cylindrical with thick duct ending at dart sac, right mucous gland sac-like, distally fused with upper portion of atrium, with thin duct ending in dart sac; both mucous gland ducts inserting independently below dart sac medial constriction; dart sac muscular, with thick walls, long, cylindrical with medial constriction, dart sac naturally folded by itself at level of constriction; dart papillae as long as distal portion of dart sac; dart sac ending at atrium level with vagina and penial complex; bursa copulatrix sac round with short to medium length duct; sac and duct of bursa copulatrix neatly delimited; vagina short; free oviduct short, enveloped by connective tissue together with bursa copulatrix; vas deferens surrounding dart sac at level of constriction before reaching peni-oviducal angle and inserting in penial complex; penial complex medium to short; flagellum thin, finger-like, ending level where vas deferens enters penial complex and epiphallus begin; proximal portion of epiphallus widening at point of entrance of vas deferens; epiphallus longer than penis externally differentiated from it; penial retractor muscle inserting in median portion of epiphallus; penis cylindrical, half-length of epiphallus, entering genital atrium level with dart sac and vagina; penial papillae short, triangular; internal wall of penis with three longitudinal pilasters; penial muscular band absent; thin, muscular sheath overlapping distal portion of penis; atrium long.

Distribution: *Epiphragmophora cryptomphala* is distributed in Chacoan biogeographic subregion in Salta and Jujuy provinces.

Remarks: *Epiphragmophora cryptomphala* is very similar in shell morphology to *E. trigrammephora*, their distributional ranges overlapping. Mostly based on shell morphologies, Parodiz (1957) and Fernández (1973) considered *E. cryptomphala* to be a subspecies of *E. trigrammephora*. However, the main differences between *E. cryptomphala* and *E. trigrammephora* are found in both the shell and genitalia. In *E. cryptomphala*, the shell umbilicus is completely overlapped or fused with the columelar side of the peristome. The shape of the aperture is not as constant as in *E. trigrammephora*, being subquadrangular to subovoidal, while in *E. trigrammephora*, all shells examined have a subquadrangular aperture. Terminal genitalia of both species are significantly different, main differences are: shape of mucous glands, length of penis and flagellum, length and thickness of bursa copulatrix duct, length of dart sac and proportion of epiphallus respect to penis. *Epiphragmophora cryptomphala* is similar to ***E. walshi*** n. sp. in that both have three peripheral bands in the shell. Also, both species live in Salta Province and are typical from xerophilic environments. *Epiphragmophora cryptomphala* differs from ***E. walshi*** n. sp. in the umbilicus that is narrower in ***E. walshi*** n. sp. and not overlapped by peristomal basal fold. ***Epiphragmophora walshi*** n. sp. is smaller in shell diameter than *E. cryptomphala*. Differences in the genitalia are mainly in the shape of mucous glands, insertion of their efferent ducts and shape and length of dart sac.

Epiphragmophora escoipensis Cuezzo, 1996
(Figs. 2B, 9A–C, 19)

Epiphragmophora escoipensis Cuezzo, 1996: 361; figs.1, 2.

Holotype: FML 14090, Argentina, Salta Prov., Chicoana Dept., Quebrada de Escoipe, 25°10'46"S, 65°45'01"W, 1,950 m, II/1993, E. Dominguez leg.

Other Material Examined:
Dissected Material: Argentina, Salta Prov.: FML 14426 A, Chicoana Dept., Quebrada de Escoipe, route 33, 25°09'26"S, 65°41'24"W, 1,650 m, 90 km from Salta city. I/1998, leg. & det. by M. G. Cuezzo. "*" FML 14091 A, PARATYPES. Chicoana Dept., Quebrada de Escoipe, 25°10'46"S, 65°45'01"W, 1,950 m, II/1993. E. Dominguez leg.
Dry Material: Argentina, Salta Prov.: FML 14427, Chicoana Dept., Quebrada de Escoipe, 1,520 m, 19/XII/2001, 25°10'03"S, 65°37'54"W, M. G. Cuezzo leg. & det.

Description:
External Features: Homogeneous light brown to yellowish body color, even color in cephalopedal region; double row of pustules in cephalic region from mantle collar to ommatophores.
Shell (Fig. 2B): Dextral, helicoidal, fragile, translucent, globose, shallow spire, small to median, 4¼ convex whorls; pale yellowish to golden; with one very thin peripheral supraequatorial band when present; holotype without peripheral band; shiny periostracum; protoconch smooth; body whorl with thin axial growth lines; suture deep impressed; aperture subovoidal; peristome whitish, thin, slightly reflexed; body whorl slightly descending towards aperture; umbilicus narrow partly overlapped by ventral columelar fold of the peristome; calcareous epiphragm.
Measurements: Holotype: D maj: 23.8 mm; D min: 21.4 mm; H: 13.8 mm; D ap: 14.1 mm; H ap: 12.5 mm.
Paratypes (n = 10): D maj: 25.2–21.0 mm (22.9 mm); D min: 20.9–18.9 mm (20.2 mm); H: 14.6–11.1 mm (13.5 mm); D. ap: 15.4–12.5 mm (13.7 mm); H ap: 12.8–10.5 mm (11.5 mm).
Jaw: Arcuate, thin, translucent; six central vertical ribs well marked with convex and concave borders projecting over jaw borders.
Pallial Complex (Fig. 9A): Pulmonary roof pale cream; kidney triangular of about 60% of pulmonary roof length; space between kidney and rectum with several thin shallow ramified veins; thick main pulmonary vein splits into two major branches close to mantle collar, with several further minor divisions forming net in distal portion of pulmonary roof.
Genitalia (Fig. 9B, C): Terminal genitalia with dart sac complex consisting of single dart sac and two mucous glands, right one globose sac-like, enveloping distal zone of vagina and dart sac, distally fused with atrial wall, left mucous gland oval ending in thin, short duct that joins with efferent duct of right gland, both inserting in medial zone of dart sac; dart sac long, muscular, cylindrical without medial constriction; dart gland thin, cylindrical located in interior of dart sac distal portion; dart sac ending at atrium level with vagina and penis; bursa copulatrix roundish with thin medial length duct; vagina shorter than penis; free oviduct short; penial complex long; flagellum thin, finger-like, ending level with where vas deferens enters penial complex and epiphallus begins; epiphallus longer, thinner than penis, externally clearly differentiated from penis; epiphallus widening in its proximal portion at point of insertion of vas deferens; penial retractor muscle inserting in medium zone of epiphallus; vas deferens surrounding dart sac; penis proximally thin, progressively widening toward distal portion; penial papillae absent; internal wall of penis with thin smooth, longitudinal folds; penis sheath, simple, muscular overlapping all penis length, distally attaching to atrium wall; atrium short.

Habitat: *Epiphragmophora escoipensis* inhabits xerophilic environments, usually adhering to cactus, under shrubs, inside crevices or below big rocks on the ground.

Distribution (Fig. 19): *Epiphragmophora escoipensis* is only known from Quebrada de Escoipe in Salta Province, northwestern Argentina. This locality is classified into the Yungas biogeographic province, although is a transition forest with some vegetational elements of the Monte biogeographic province.

Remarks: In the original species description, only one mucous gland (the left one) of the terminal genitalia was detected. The specimens used to describe the new taxon probably had the second gland collapsed, as a result of the fixative solution (formaldehyde) used. In the present study, the dissection of several other specimens showed that the two mucous glands are indeed present in *E. escoipensis* genitalia, one of them as a sac enveloping the basal portion of the genitalia.

Epiphragmophora estella (d'Orbigny, 1835) (Fig. 2C)

Helix estella d'Orbigny, 1835: 241.

Syntypes: MNHN, Bolivia, Valle Grande, close to the village of Tasajos. BMNH, Bolivia, Chuquisaca. BMNH, Bolivia, Valle Grande, BMNH, Bolivia, Sicasica.

Other Material Examined:
Dry Material: Bolivia: MNHN, Santa Cruz Dept., Valle Grande. FML, Cochabamba Dept., Punata Prov. Cerro Tuti, 4,000 m, 17/I/1950. R. Zischka leg. W. Weyrauch det. FML, Cochabamba Dept., Cozomi, 3,200 m, 30/XII/1949. R. Zischka leg. W. Weyrauch det. MLP unnumbered, Cochabamba Dept., Aguirre, 3,200 m, ex col. Hylton Scott.

Description:
Shell (Fig. 2C): Dextral, helicoidal, globose, fragile not translucent, with conical spire, medium size, 4¾ convex whorls; pale cream to yellowish; with peripheral equatorial thin, darker brown band; protoconch smooth; teleoconch surface malleated with diagonal ribs on body whorl; suture deep impressed; aperture elliptical to subcircular, peristome whitish, thin, slightly expanded; body whorl distinctly descending towards aperture; umbilicus narrow partly overlapped by ventral columelar fold of peristome.
Measurements: Syntypes (n = 2): D maj: 30.0 mm, 32.2 mm (31.1 mm); D min: 26.8 mm, 27.9 mm (27.3 mm); H: 17.9 mm, 19.9 mm (18.9 mm); D ap: 18.6 mm, 19.8 mm (19.2 mm); H ap: 15.2 mm, 17.2 mm (16.2 mm).

Distribution: The distribution of this species is restricted to the departments of La Paz, Santa Cruz, and Chuquisaca in Bolivia.

Remarks: When d'Orbigny described the present species, he mentioned the existence of three varieties, called by him "A, B and C", from three different localities in Bolivia. Variety "A", was called "reticulata, imperforata", which has the most globose shell, is smaller in diameter (13–24 mm) and was found in the localities of Inquisivi (16°58'S, 67°10'W) and Caranavi (17°13'S, 66°57'W), both from La Paz Dept. Variety "B", called "substriata, perforata, magna", is the largest in shell diameter (20–38 mm) and it was found in Chuquisaca Dept., Bolivia. Variety "C", called "laevignata, perforata, depressa", has the most depressed shell among the three varieties and an open umbilicus, found in the village of Tasajos (18°08'S, 64°15'W) in Valle Grande, Santa Cruz Dept., Bolivia. The major differences between them are shell diameter, shell sculpture and degree of closure of the umbilicus. The shell description provided here corresponds to the "varieté B, substriata, perforata, magna", of d'Orbigny species description, the biggest variety of *E. estella*. Photographs of the syntypes deposited at The Natural History Museum, London, corresponding to Varieties A and C were examined, corresponding also to this species. However, only the availability of anatomical information will clarified the status of d'Orbigny varieties.

Epiphragmophora guevarai Cuezzo, n. sp. (Figs. 7A, 18A–C, 21)

Holotype: FML 14420 A (alcohol-preserved specimen), Argentina, Santiago del Estero, 20 km S from Ojo de Agua, 29°19'32"S, 63°48'52"W, 570 m 30/XII/2002, M. G. Cuezzo leg.
Paratypes: FML 14420 (dry material, type locality); MLP unnumbered (type locality). FML 14766, near Ojo de Agua, km 922 on the highway from Tucumán to Córdoba, 24/XII/1967, leg. W. Weyrauch.

Other Material Examined:
Argentina, Cordoba Prov.: FML 14717, 5 km N from Villa María, 440 m, 25/XII/1967, W. Weyrauch leg. FML 10989, Dean Funes Dept., Cerro de La Cruz, Sierra de Orcosumi, 800 m, J. Durango leg.

Diagnosis: Shell with three thin, continuous peripheral bands, central one distinctly delimited, the others more tenuous; shell surface with thin, delicate axial growth-lines; two unequal mucous glands in terminal genitalia, both basally attached to atrium inserting through separated efferent ducts into distal dart sac; dart sac ending at atrium level with vagina and penial complex; bursa copulatrix duct extremely short but thin, basally wider; penis proximally globose abruptly becoming thinner towards distal portion; atrium globose, not enveloped by mucous gland.

Etymology: The present species is named after Dr. Ernesto Guevara de la Serna, for his life and ideals.

Description:

External Features: Animal color uniformly light gray with double dorsal line of pustules in cephalic region.

Shell (Fig. 7A): Dextral, subglobose, fragile, not translucent, spire medium elevated, conical, medium size with 4 convex whorls; pale brown; body whorl with three thin, continuous peripheral bands, central one darker and well marked close to aperture, dorsal and ventral bands wider at body whorl, both tenuous close to aperture, darker central band overlapping with suture; protoconch smooth; teleoconch surface with thin axial growth-lines; suture shallow; aperture subcircular with white, thin, slightly expanded peristome; space between terminal point of peristomal lips narrow with shallow callous present in some specimens; body whorl descending behind aperture; umbilicus wide, deep, partly overlapped with peristomal lip.

Measurements: Holotype: D maj: 24.6 mm; D min: 21.9 mm; H: 14.7 mm; D ap: 14.0 mm; H ap: 13.0 mm.

Paratypes (n = 12): D maj: 23.7–27.2 mm (25.1 mm); D min: 20.3–22.6 mm (21.7 mm); H: 13.3–15.3 mm (14.2 mm); D ap: 13.6–16.6 mm (14.9 mm); H ap: 12.3–14.6 mm (12.8 mm).

Jaw: Arcuate, orange, thin and translucent with three central wide ribs.

Pallial Complex: Pulmonary roof pale cream without darker spots; kidney length about 60% of pulmonary roof length; space between kidney + primary ureter and rectum + secondary ureter traversed by several thin ramified veins; thick main pulmonary vein splitting into two thinner main branches close to mantle collar.

Genitalia (Fig.18A–C): Terminal genitalia with single dart sac and two unequal mucous glands, right gland smaller, roundish, located between dart sac and vagina, distally fused with atrium wall, with thin, short duct; left mucous gland oval with wider short efferent duct, basally attached to atrium; both efferent ducts opening into dart sac contiguous, not fused; dart sac long, muscular, cylindrical, with medial constriction, mucous gland ducts inserting below constriction; dart papillae cylindrical, as long as distal portion of dart sac; dart sac inserting in atrium level with vagina and penial complex; bursa copulatrix sac oval, with thin, short duct distally slightly wider; sac and duct of bursa copulatrix neatly delimited; vagina shorter than penis; penial complex short; flagellum short, finger-like; epiphallus cylindrical to globose in proximal portion where vas deferens inserts, shorter than penis, externally differentiated from it; penial retractor muscle inserting in median portion of epiphallus, basally wider; penis proximal portion globose to cylindrical, abruptly getting thinner towards distal portion, penis twice epiphallus length, entering at genital atrium level with dart sac and vagina; penial papillae short, located in proximal penis; internal wall of penis with thin zigzag folds in all its length; penis sheath simple, muscular, overlapping distal portion of penis and proximally attaching to vas deferens by connective tissue and muscular strands; penial muscular band absent; atrium medium in length, globose, not enveloped by mucous gland.

Distribution (Fig. 21): Southern Santiago del Estero to Northern Cordoba provinces, Argentina. The present species is an habitant of the Chacoan biogeographic subregion.

Remarks: The present species is similar to *E. trifasciata* Fernández & Rumi. Some of the dry material in museum collections has been misidentified as *E. trifasciata* or as *E. trenquelleonis*. ***Epiphragmophora guevarai*** n. sp. differs from *E. trifasciata* mainly in shell characters: 1. Smaller shell size, 2. Peripheral bands thinner, median neatly delimited, 3. Shell sculpture consisting in thin stria instead of strong growth-ridges and both shell and animal color. Concerning the terminal genitalia, main differences are: (1) duct of bursa copulatrix is short but thinner and basally wider, bursa copulatrix is oval instead of round as in *E. trifasciata*, (2) flagellum shape and length, being finger-like instead of triangular, (3) left mucous gland oval in shape and smaller than in E. *trifasciata* inserting into dart sac separately from the thinner efferent duct of the other mucous gland, (4) penial morphology, especially penis shape with proximal portion globose abruptly diminishing in diameter towards atrium. Both species have contiguous distributions. ***Epiphragmophora guevarai*** differs from *E. trenquelleonis* in having a smaller shell size, different shell and body color, three peripheral bands, and a genital morphological pattern completely different. General shell shape is similar in both species.

Epiphragmophora hemiclausa Hylton Scott, 1951
(Figs. 2D, 10A–D, 19)

Epiphragmophora semiclausa Hylton Scott, 1951: 257, fig. 3.2, *non E. semiclausa* (Martens, 1869).
Epiphragmophora hemiclausa Parodiz, 1957: 30, *nom. nov*.
Epiphragmophora hemiclausa – Fernandez & Rumi, 1984 b: 239.

Holotype: MLP unnumbered, Argentina, Catamarca Prov., Cuestecilla de La Viña, 9/III/1950, Gladys Biraben leg.

Other Material Examined:
Dissected Material: Argentina, Catamarca Prov.: *FML 14410A, Paclin Dept., Route 38, 5 km to the S of provincial limit, La Viña, 28°02'20"S, 65°35'48"W, 600 m, 25/I/1997, M. G. Cuezzo & E. Dominguez leg. FML 14424, Andalgalá Dept., Alto de las Juntas, 15 km N of Rodeo, II/1997, P. Fidalgo, leg. *FML 14425, Paclin Dept., La Viña, 600 m, 12/XI/2001, M. G. Cuezzo leg. Argentina, Tucumán Prov.: FML 14403A, Lules Dept., margins of Lules river, 2,000 m E. Dominguez leg.
Dry Material: Argentina, Catamarca Prov.: FML 454, El Alto, 950 m, 13/XI/1958, Z. Ajmat leg., W. Weyrauch det. FML 744, Cuesta El Totoral, 950–1,200 m, 6/VIII/1967, W. Weyrauch leg & det. FML 775, El Totoral, 950–1,200 m, 16/XII/1963, W. Weyrauch leg.& det. FML 562, El Rodeo, I/1958, R. Golbach leg., W. Weyrauch det. FML 752, Near La Merced, subtropical rainforest, 6/VIII/1967, W. Weyrauch leg. & det. FML 11053, Between La Viña and the river Huacra, 550–600 m, 16/XII/1963, W. Weyrauch leg & det. FML 773, El Durazno, 850–900 m, W. Weyrauch leg. & det. FML 655, Ambato Dept., Sierra de La Puerta, 2/I/1939, Pierotti leg. FML 1245, Cuesta de La Viña, 700 m, 13/VIII/1967, W. Weyrauch leg. & det. MLP 4279, Cuesta de La Viña, Gladys Birabén leg., 9/III/1950. MACN 24251, Cuesta La Viña (Paratypes). MLP unnumbered, Sumampa, Biraben-Scott leg., 9/III/1950, ex collection Hylton Scott. Argentina, Tucumán Prov.: FML 801, on road from Tapia to Raco, 900 m, under rocks, 14/II/1964, W. Weyrauch leg. & det.

Description:
External Features: Uniform light yellowish, with double dorsal line of pustules in cephalic region.

Shell (Fig. 2D): Dextral, helicoidal, globose, semisolid to fragile, with conical spire; small to medium size, 4¼ convex whorls; light yellowish to pale brown, with single peripheral equatorial, dark brown even band more evident in body whorl periphery but running over suture of spire; protoconch smooth; teleoconch surface with thin axial growth lines and body whorl malleated, both dorsal and ventrally; suture deep impressed; aperture subcircular, peristome whitish, thin, slightly expanded, reflexed; body whorl distinctly descending towards aperture; umbilicus narrow, partly overlapped by ventral columelar fold of umbilicus; holotype with peristome totally overlapped by peristome, not fused.
Measurements: Holotype: D maj: 27.5 mm; D min: 23.2 mm; H: 14.6 mm.
Paratypes (n = 8): D maj: 21.9–24.0 mm (22.7 mm); D min: 18.921.2 mm (20.3 mm); H: 11.0–13.6 mm (12.8 mm).
Shell of the specimens dissected from Catamarca Province (n = 12): D maj: 24.6 mm; D min: 22.5 mm; H: 15.0 mm.
Jaw: Arcuate, thin, translucent, with three vertical, central ribs; concave and convex borders of ribs projecting at jaw edges.
Pallial Complex (Fig. 10D): Pulmonary roof with great number of minor transverse veins; kidney thin, triangular, with half pulmonary total roof length; thick main pulmonary vein splitting into two main branches close to mantle collar.
Genitalia (Fig. 10A–C): Terminal genitalia with dart sac complex consisting of single dart sac and two unequal mucous glands, right one round-shaped and left one elongate oval-shaped, both with ducts inserting independently in middle zone of dart sac; dart sac muscular, short, widening at base, inserting in distal portion of vagina; internal wall of dart sac with uniform longitudinal bands; dart papillae present, short, in distal portion of dart sac; bursa copulatrix elongate-oval, running towards middle zone of spermoviduct; duct of bursa copulatrix long, slightly swollen at base; vagina long (as long as penis); free oviduct short; penial complex with thin flagellum, finger-like; transition between epiphallus and penis not evident externally; epiphallus slightly longer to double penis length; proximal portion widening at vas deferens point of insertion; internal walls bearing series of slender longitudinal pleats; penis retractor muscle, thick, wide, inserting in middle epiphallus; wall sculpture of proximal penis with thin zigzag longitudinal

pleats, distal portion with thin straight longitudinal bands; small penial papillae present; penial sheath muscular, thin, proximally translucent, penis completely enveloped by penial sheath; vas deferens not surrounding dart sac; penis and vagina entering side by side atrium, distally to dart sac complex; penial muscular band present.

Habitat: *Epiphragmophora hemiclausa* is usually found under pieces of tree logs on the floor or among dead leaves.

Distribution (Fig. 19): Distributed in Argentina, its area of distribution corresponds to the Yungas biogeographic province that extends in Catamarca and in central and southern Tucumán Province.

Remarks: The present species was originally described by Hylton Scott (1951) as *Epiphragmophora semiclausa*, but later the name was changed to *E. hemiclausa* by Parodiz (1957) because *Lysinoe semiclausa* Martens, 1869, was classified into the genus *Epiphragmophora* and therefore the species name was preoccupied. *Epiphragmophora hemiclausa* is similar to *E. argentina* in shell morphology, but it is usually smaller in diameter and presents a perspective umbilicus instead of being imperforated. It also differs in characters of the genitalia: shape of mucous glands and dart sac, length of bursa copulatrix duct.

Epiphragmophora hieronymi Doering, 1874
(Figs. 3A, 9D–F, 21)

Helix Epiphragmophora hieronymi Doering, 1874: 168.
Helix Aglaja Yocotulana – Doering, 1874: 446.
Helix Hieronymi – Kolbelt, 1878, 5: 146.
Epiphragmophora hieronymi – Parodiz, 1957: 29.
Epiphragmophora (*Epiphragmophora*) *hieronymi* – Zilch, 1959–1960: 655.
Epiphragmophora hieronymi – Fernández & Rumi, 1984b: 240.

Lectotype: SMF 7712, Catamarca, Quebrada del Tala, Hieronymus leg.
Paralectotypes: MACN 9127, Argentina, Catamarca, Sierra de Belén, close to Yocotula.

Other Material Examined:
Dissected Material: Argentina, Tucumán Prov.: FML 14414 A, Tafi del Valle Dept., between Amaicha and El Infiernillo close to Ampimpa, 26°38'06"S, 65°50'05"W, 2,610 m, 20/V/2003, M. G. Cuezzo leg. & det. Argentina, Catamarca Prov.: FML 14421 A Andalgalá Dept., Las Estancias, El Pucará, 2,040 m, 19/IV/2003, 27°42'21"S, 66°00'12"W, M. G. Cuezzo leg. & det. FML 14444 A, Tinogasta Dept., Cuesta de Zapata, between Belén and Tinogasta, 27°53'53"S, 67°22'23"W, 1,710 m, 28/XI/2003, Ituarte, C. leg.
Dry Material: Argentina, Tucumán Prov.: FML 212, Tafi del Valle Dept., Tafi, 26/IX/1946, Haywart, R. leg., W. Weyrauch det. FML14422, Tafi del Valle Dept., Route 307, close to Amaicha, 26°40'49"S, 65°48'46"W, 2,850 m, XII/2001, M. G. Cuezzo leg. & det. FML 14441, Tafí del Valle Dept., Amaicha, close to Castillo de las Brujas, 26°38'04"S, 65°52'10"W, 2,245 m, 15/IX/2003, D. Garcia Lopez leg. FML 14720, Tafi del Valle Dept., Route 307, between Amaicha and El Infiernillo, 26°38'18"S, 65°49'30"W, 2,650 m, M. G. Cuezzo leg. FML 14731, Tafi del Valle Dept., Quilmes, ruins of Quilmes, 26°27'41"S, 66°02'19"W, 2,000 m, 24/III/2005, M. G. Cuezzo leg. & det. Argentina, Catamarca Prov.: FML 135, Andalgalá Dept., La Mina, 13/VIII/1929, W. Weyrauch det. FML 14370, Dique las Pirquitas, 21/I/1968, W. Weyrauch leg. & det. FML 14423, Route 60, Quebrada de La Cébila, between Chumbicha and Mazán, IV/1998, M. G. Cuezzo leg. & det. FML 972, Cuesta de Las Chilcas, 24/X/1933, Schreiter, R. leg., W. Weyrauch det. FML 10897, Cuesta de Zapata, 1,500–1,850 m, 28/XI/1967, W. Weyrauch leg. & det. FML 14726, Andalgalá Dept., Cuesta Mina Capillitas, 27°29'54"S, 66°22'53"W, 1,630 m, 29/XI/2003, M. G. Cuezzo & C. Ituarte leg. FML 14727, Belén Dept., Farallón Negro, 27°17'37"S, 66°40'36"W, 2,500 m, 26/XI/2003, M. G. Cuezzo leg. & det. FML 14728, Belén Dept., Cuesta de Belén, 5 km N of Belén, 27/I/2003, 27°37'07"S, 67°01'19"W, 1,310 m, M. G. Cuezzo leg. & det. MACN 9127. MACN 19618, Fiambalá Mountains, E. Kittl col., 1930. MACN 9127, Sierra de Belén, close to Yocotula. MLP 3663, Mutquib, O. Ferraris leg. V/1953. MLP 1293, Los Angeles, 1,800 m MLP 4237, El Rodeo. MLP 4238, Las Trancas. MLP unnumbered, Las Pirquitas, ex collection Hylton Scott, Palma, A. leg., X/1950. Argentina, La Rioja Prov.: MLP 4235: Peñas. Biraben, M. Ex Hylton Scott collec-

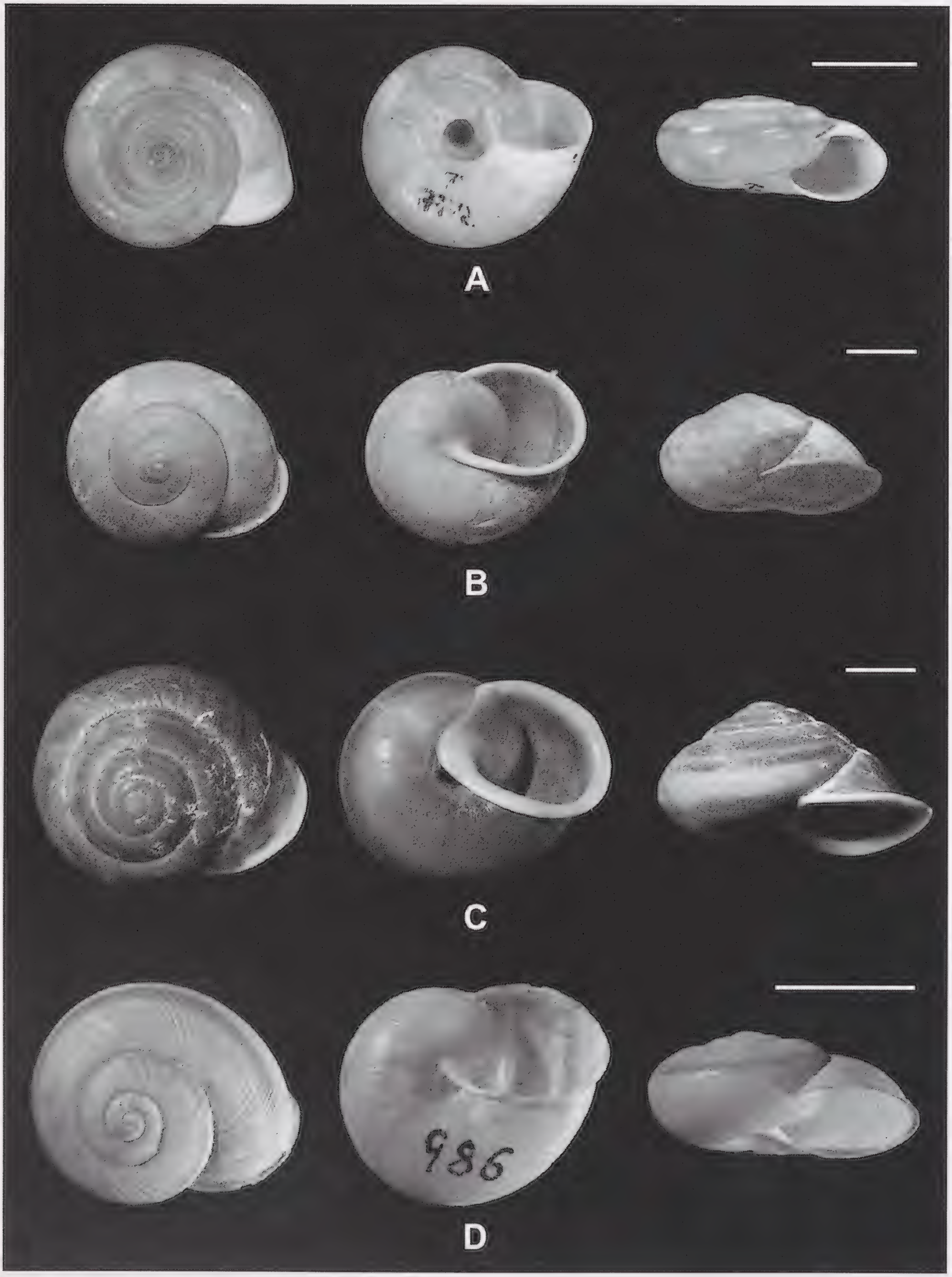


FIG. 3. Shells in dorsal, ventral and lateral view. A: *Epiphragmophora hieronymi* (SMF 7712, lectotype). Scale bar = 15 mm; B: *Epiphragmophora jujuyensis* (MLP, holotype). Scale bar = 10 mm; C: *Epiphragmophora oresigena* (MNHN, syntype). Scale bar = 10 mm; D: *Epiphragmophora parodizi* (FML 986 a, holotype). Scale bar = 20 mm.

TABLE 1. Main characters differencing the species: *E. hieronymi*, ***E. quirogai***, *E. puella*, and *E. villavilensis*.

Characters	*E. hieronymi* (Doering)	*E. puella* Hylton Scott	***E. quirogai*** New Species	*E. villavilensis* Parodiz
Animal body color	Pale cream to pale grey	Not known	Black	Pale cream
Sculpture of body whorl	Axial growth lines	Granules and wrinkles	Axial growth lines and peripheral lines to malleated	Axial growth lines and peripheral thin lines
Suture	Shallow	Deeply marked	Deeply marked	Deeply marked
Shell Shape	Flat, low spire	Flat, low spire	Subglobose, low spire	Subglobose, depressed
Number of peripheral bands	One	None	None	None
Body whorl periphery	Convex	Equatorially subcarinated	Convex	Supraequatorially subcarinated
Peristomal lip	Thin, slightly expanded	Expanded	Thick, slightly reflexed	Thick, slightly reflexed
Aperture	Subcircular to oval	Subcircular to oval	Subcircular	Subquadrate
Internal sculpture of aperture and wall	With pustules	With pustules	With pustules	With pustules
Proximal portion of dart sac (until constriction)	Usually longer than distal portion	Not known	Usually longer than distal portion	-
Mucous gland	Incompletely surrounding basal dart sac and vagina	Not known	Incompletely surrounding basal dart sac and vagina	Completely surrounding basal dart sac plus vagina
Flagellum	Thin, finger-like, as long as epiphallus lenght	Not known	Thin, finger-like, less than half epiphallus lenght	Thin, finger-like, as long as epiphallus lenght
Epiphallus lenght	As long as penis	Not known	Double lenght of penis	Half penis lenght

tion. 13/XII/1951. FML 11044, La Torre, on road to Patquia, km 75, 1,000 m, 1/IV/1966, R. Herbst leg. W. Weyrauch det. Argentina, Formosa Prov.: MLP unnumbered, Estero Bellaco. Pierotti leg. (ex coll. Hylton Scott). Argentina, San Luis Prov.: MLP 4309, Carolina, 1,600 m 25/X/1970. Viana leg. (ex collection Hylton Scott). Argentina, Salta Prov.: FML 14730, Metán Dept., 6 km from national route 9 on margins of road to Cabra Corral, 25°07’33"S, 65°04’16"W, 837 m, 9/II/2005, M. G. Cuezzo leg. & det.

Description:

External Features: Body color varies from grayish to pale cream.

Shell (Fig. 3A): Dextral, helicoidal, semisolid to fragile, with low conical to flat spire; small to medium size, 4¼ convex whorls; pale yellowish to whitish; single peripheral supra-equatorial, brownish, thin, even band running in body whorl periphery and on suture between third and fourth whorl; protoconch smooth; teleoconch surface with thin axial growth lines; suture shallowly impressed; aperture subcircular, peristome usually whitish, thin, slightly expanded; wall sculpture inside aperture with small irregularly distributed pustules; body whorl not descending towards aperture or scarcely descending; umbilicus wide, perspective not overlapped by ventral columelar fold of the peristome.

Measurements: Lectotype: DM: 18.4 mm; H: 8.6 mm.
Specimens from Argentina, Tucumán, Tafi del Valle Dept. (n = 25): DM: 24.6–10.4 mm (22.4 mm); D min: 21.4–18.5 mm (19.7 mm); H: 11.0–9.1 mm (9.5 mm); D ap: 12.3–10.0 mm (11.0 mm); H ap: 10.6–8.8 mm (9.7 mm).

Jaw: Small, translucent; four to five vertical ribs in center of jaw; borders of ribs projecting at convex and concave edges of jaw.

Pallial Complex: As described in Cuezzo (1997).

Genitalia (Fig. 9D–F): Terminal genitalia with dart sac complex consisting of single dart sac and two unequal mucous glands; right mucous gland sac-like enveloping distal zone of vagina and dart sac, distally fused with atrial wall; left mucous gland oval-elongate, ending in efferent duct that joins and fuses with thinner duct of right gland, both forming single efferent duct inserting in medial zone of dart sac below its constriction; dart sac muscular, long, cylindrical, with a constriction dividing the organ in two portions; dart sac proximal portion longer than distal portion; thin, internal dart gland located in distal portion of dart sac; dart sac ending in atrium level with vagina and penis; bursa copulatrix round, with thick, short duct; vagina very short almost completely covered by mucous gland; flagellum thin, finger-like, short, ending level where vas deferens enters penial complex and epiphallus begins; flagellum as long as epiphallus length; internally, proximal epiphallus with thick pilaster beginning at point of insertion of vas deferens and progressively tapering towards distal epiphallus; epiphallus as long as penis, proximally broad and progressively becoming thinner; penial retractor inserting close to vas deferens entering at proximal epiphallus; vas deferens forming a loop around penial retractor before inserting in epiphallus; penis cylindrical entering at genital atrium level with dart sac and vagina; penial papillae short, triangular; internal wall of penis with thin delicate longitudinal pleats; penis sheath muscular, thick, overlapping terminal portion of penis, distally fused to atrium wall; atrium long.

Habitat: Snails of this species are found under rocks in contact with the soil or inside rock crevices. Usually not found adhering to rocks, generally grounded in soil under rocks. Alive specimens are difficult to find, probably living most of their life inside deep crevices to avoid water loss.

Distribution (Fig. 21): *Epiphragmophora hieronymi* is found in Tucumán, Salta, Catamarca, Formosa, San Luis and La Rioja provinces, Argentina. It inhabits the Monte biogeographic province, and in Pre-Puna generally in places of high altitude (above 1,000 m). In Salta and Formosa, Chacoan biogeographic subregion at lowers altitudes.

Remarks: The epiphragm in this species is calcareous-like, white and thick, never transparent. It is very conspicuous compared to the epiphragm of the rest of the species examined. Some differences in epiphallus lengths were registered in specimens of different localities and could be assumed as individual variations. In specimens dissected that were previously fixed in formaldehyde or that have several years of being collected and preserved in malacological collections, the mucous glands were collapsed and the tissues were hardened, especially the mucous gland surrounding the distal genitalia. Because of this artificial modification, the mucous gland seems to be a thick sheath that surrounds terminal genitalia similar to the one present in some Cepoliinae instead of a soft glandular tissue of a mucous gland with similar position. Main shell and genitalia differences among *E. hieronymi, E. puella,* ***E. quirogai*** n. sp. and *E. villavilensis* are detailed in Table 1.

Epiphragmophora jujuyensis Hylton Scott, 1962
(Figs. 3B, 11A–C, 19)

Epiphragmophora jujuyensis Hylton Scott, 1962: 107.

Epiphragmophora jujuyensis – Fernández & Rumi, 1984b: 241.

Holotype: MLP unnumbered, Argentina, Jujuy, Las Capillas, 4/XII/1951.

Paratypes: MACN 16234, Argentina, Jujuy.

Other Material Examined:

Dissected Material: Argentina, Salta Prov.: FML 14513 A, Anta Dept., National Park “El Rey”, Aguas Negras, 25°15’36"S, 64°55’15W, 645 m, 23/III/1999, M. G. Cuezzo leg. & det. FML 14514 A, Anta Dept., National Park “El Rey”, Cerro Maldonado, 1,400 m, 24°43’27"S, 64°38’46"W, 24/III/1999, M. G. Cuezzo leg. & det., FML 14515 A, Orán Dept., San Andrés, 23°05’50"S, 64°46’10"W, 1,090 m, 16/XII/1999, E. Dominguez leg. FML 14512 A Orán Dept., on road to San Andrés, junction between San Andrés river with Seco river, 23°05'50"S, 64°46'01"W, 1,000 m, E. Dominguez leg. FML 14516 A, Anta Dept., National Park "El Rey", margins of La Sala river, 24/III/1999, M. G. Cuezzo leg.

Dry Material: MLP unnumbered, Top of the mountains Maldonado, National Park "El Rey", Brown, A. 15/VII/1979. MLP unnumbered, Burela, Birabén, M. Argentina, Jujuy Prov.: FML 960, La Caldera Dept., on road between Salta and Jujuy, km 1650, 1,400 m, 29/II/1964, W. Weyaruch leg. & det. FML 806, Manuel Belgrano Dept., Las Capillas, 36 km NE from San Salvador de Jujuy, 1,400 m, 25/II/1964, W. Weyrauch leg. & det. FML 893, Manuel Belgrano Dept., Los Perales, 1,300 m, 25/II/1964. FML 14517, Ledesma Dept., National Park Calilegua, margins of the river "tres cruces", 23°41'36"S, 64°52'08"W, 1,100 m, 2/IV/2004, M. G. Cuezzo leg. FML 14729, Santa Bárbara Dept., Villa Monte, Finca Portal del Cerro, 24°05'S, 64°23'W, 1,068 m, 1/III/2005, F. C. Cuezzo leg.

Description:

External Features: Body dark brown, with tentacles usually darker; well-marked dorsal row of pustules in cephalic region passing between ommatophores; lung spots visible through shell by transparency, appearing as shell spots in live specimens.

Shell (Fig. 3B): Dextral, helicoidal, globose, solid, with elevated conical spire; medium to large size, 4½ convex whorls; dark to light brown, with thin, peripheral supraequatorial dark brown band more evident in body whorl; protoconch smooth; teleoconch surface malleated with diagonal ribs on body whorl; suture deeply impressed; aperture elliptical, peristome whitish slightly reflected, with callous lower margin; body whorl distinctly descending towards aperture; umbilicus narrow, overlapped by ventral columelar fold of peristome in holotype; some specimens with umbilicus not totally overlapped by peristome.

Measurements: Holotype: Argentina, Jujuy, Las Capillas, D maj: 32.4 mm; D min: 29.7 mm; H: 19.1 mm.

Specimens from Argentina, Salta, Parque Nacional "El Rey" (n = 10): D maj: 30.7–35.0 mm (32.6 mm); D min: 27.4–31.2 mm (29.2 mm); H: 18.3–21.6 mm (19.5 mm).

Specimens from Argentina, Jujuy, Parque Nacional "Calilegua" (n = 8): D maj: 38.3–44.1 mm (42.0 mm); D min: 33.0–39.0 mm (36.0 mm); H: 21.3–27.7 mm (23.9 mm).

Jaw: Corneous, strong; two strong vertical ribs in central portion.

Pallial Complex: Lung roof densely mottled with black spots when viewing externally; kidney triangular, as long as half of pulmonary roof length; main pulmonary vein splitting into three major veins and several minor longitudinal veins.

Genitalia (Fig. 11A–C): Terminal genitalia with dart sac complex consisting of single dart sac and two mucous glands on opposite sides; mucous glands oval, pear-shaped; right mucous gland (dorsal view) slightly shorter than left; both mucous glands opening above medial constriction of dart sac via individual efferent ducts independent from each other; dart sac cylindrical, muscular, with medial constriction, slightly longer than bursa copulatrix; vas deferens surrounding dart sac at level of dart sac constriction; proximal dart sac internal wall with longitudinal folds, distal internal dart sac with cylindrical dart papilla; dart sac opening into atrium; vagina cylindrical, short; free oviduct cylindrical thinner than vagina; bursa copulatrix with short, thick duct; bursa sac not sharply differentiated from duct; penial complex consisting of flagellum, epiphallus and penis; flagellum finger-like, short, curved, ending where vas deferens enters penial complex and epiphallus begins; epiphallus long, with internal longitudinal pleats; proximal portion of epiphallus broadening at point of insertion of vas deferens; penial retractor muscle inserting in middle zone of epiphallus; vas deferens forming loop around penial retractor; penis longer than epiphallus, entering at genital atrium level with respect to vagina and dart sac openings; penial papilla short, triangular, located in proximal portion of penis; proximal penis wall with convoluted zigzag glandular bands close to penial papillae; distal penis wall with longitudinal straight bands; penis sheath thin, translucent, overlapping distal portion of penis; genital atrium large, with inner wall smooth.

Habitat: Usually found under leaf litter and fallen tree trunks in humid forests.

Distribution (Fig. 19): *Epiphragmophora jujuyensis* is known from Jujuy and Salta provinces, northwestern Argentina. This species is a typical habitant of the cloud rainforest or Yungas biogeographic province although it is not easily found.

Remarks: Holotype measurements in the original description of the species (Hylton Scott, 1962) are not coincident with the real measurements of the holotype deposited at La Plata Museum, Argentina. There is a great variability in shell diameter among populations of Jujuy and Salta localities, the specimens from Calilegua National Park, Jujuy Province, being bigger.

Epiphragmophora oresigena (d'Orbigny, 1835)
(Fig. 3C)

Helix oresigena d'Orbigny, 1835: 239: pl. 24.

Syntypes: MNHN, Bolivia, Yungas.
Type Locality: Provincia Yungasensi, republica Boliviana, prés du confluent du Rio Meguilla et du Rio de La Paz.

Other Material Examined:
Dry Material: FML 399, BOLIVIA, La Paz Dept., Yungas Prov. Coroico, 1,700 m, in a coffee plantation. 25/I/1966, E. de la Sota leg.

Description:
Shell (Fig. 3C): Dextral, helicoidal, globose, very solid with conical, obtuse spire; large size, 5 $^1/_3$ convex whorls; dark to light brown; three peripheral dark brown bands more evident in body whorl, ventral pigmented band usually wider, central and dorsal supra-equatorial, only central and dorsal bands in spire; protoconch smooth; teleoconch surface malleated with diagonal ribs on body whorl; suture deep impressed; aperture subquadrangular, peristome thick, white, reflected; narrow, shallow fold in parietal wall between margins of peristome; body whorl abruptly descending behind aperture; umbilicus narrow partly overlapped by ventral columelar fold of peristome.
Measurements: Syntypes (n = 2): D maj: 37.9 mm, 41.9 mm (39.9 mm); D min: 32.7 mm, 35.3 mm (34.0 mm); H: 23.3 mm, 26.7 mm (25.0 mm); D ap: 21.4 mm, 25.7 mm (23.5 mm); H ap: 18.0 mm, 21.1 mm (19.5 mm).

Distribution: Known from few localities in La Paz Dept., Yungas Province, Bolivia (Breure, 1973).

Epiphragmophora parodizi Fernández & Rumi, 1984
(Figs. 3D, 11D, E, 19C, 20)

Epiphragmophora parodizi Fernández & Rumi, 1984b: 242; pl. 5, figs. 7–9.

Holotype: FML 986a, Argentina, Tucumán Prov., Tafi del Valle Dept., Infiernillo. W. Weyrauch leg., 5/IV/1965.

Other Material Examined:
Dissected Material: Argentina, Tucumán Prov.: FML 14395A, Trancas Dept., on road to Gonzalo. 26°19'35"S, 65°32'21"W, 1,360 m, 7/V/2002, M. G. Cuezzo leg. & det. FML 14396A, Trancas Dept., on road from San Pedro de Colalao to Gonzalo, close to Rearte, 26°19'35"S, 65°32'21"W, 13/XI/2000, 1,460 m, M. G. Cuezzo leg. & det. FML 14389A, Trancas Dept., close to San Pedro de Colalao, 15/X/1996, M. G. Cuezzo & F. Romero leg. FML 14397A, Trancas Dept., close to the bifurcation to Hualinchay, 26°21'05"S, 65°32'25"W, 1,450 m, 13/XI/2000. M. G. Cuezzo leg. & det.
Dry Material: Argentina, Tucumán Prov.: FML 889, Tafi del Valle Dept., El Infiernillo, 3,100 m, 10/III/1964. A. Willink leg. FML 14388, Tafí del Valle Dept., road between Tafí and El Rincón behind Loma Pelada, 26°56'20"S, 65°46'31"W, 26/XI/2002, M. G. Cuezzo & E. Dominguez leg. FML 14394, Trancas Dept. on road to Gonzalo before Los Rearte, km 31. 15/X/1996. M. G. Cuezzo & F. Romero leg. FML 14398, Trancas Dept., South of San Pedro de Colalao, I/1986, M. G. Cuezzo det. FML 230, Tafi del Valle Dept., Tafi del Valle, 2,000 m, 27/X/1947, R. Golbach leg. FML 14739, Tafi del Valle Dept., El Mollar, 2,000 m, XI/1998, M. G. Cuezzo & E. Dominguez leg. FML 14740, Trancas Dept., on road to Gonzalo, 7/V/2002, 1,400 m, M. G. Cuezzo leg. & det.

Description:
External Features: Body light brown with suprapedal dark brown longitudinal band extending from head to tail; suprapedal dark band thicker close to tail.
Shell (Fig. 3D): Dextral, helicoidal, semisolid to fragile, with conical shallow spire; small size, 4 $^1/_4$ convex whorls; light yellowish to golden brown, with peripheral supra-equatorial, dark brown band progressively becoming thicker towards body whorl, band running above suture in spire; protoconch

smooth; teleoconch with regularly distributed axial ribs separated at regular intervals; suture deep impressed; aperture subcircular to transverse oval; peristome whitish, thick, reflexed; body whorl distinctly descending towards the aperture; umbilicus narrow, partly overlapped by ventral columelar fold of peristome.

Measurements: Holotype: D maj: 19.7 mm; D min: 17.0 mm; H: 9.6 mm; D ap: 12.2 mm; H ap: 9.4 mm.

Specimens from Trancas Dept. (n = 18): Dmaj: 23.9–18.7 mm (20.5 mm); D min: 20.9–16.4 mm (16.8 mm); H: 13.1–9.6 mm (11.2 mm); D ap: 14.5–11.2 mm (12.3 mm); H ap: 11.6–9.0 mm (10.2 mm).

Specimens from Tafí del Valle Dept. (n = 7): D maj: 23.3–19.4 mm (20.8 mm); D min: 20.4–17.5 mm (18.2 mm); H: 11.8–9.5 mm (10.3 mm); D ap: 14.3–11.9 mm (12.8 mm); H ap: 11.2–9.6 mm (10.3 mm).

Jaw: Thin, translucent; specimens with either wide, shallow plaques or four ribs in center of jaw.

Genitalia (Fig. 11D, E): Terminal genitalia with dart sac complex consisting of single dart sac and two mucous glands; right mucous gland sac-like, enveloping distal zone of vagina and dart sac, distally fused with atrial wall; left mucous gland oval-elongated, basally wider opening into broad duct that joins with thinner duct of right gland, both ducts inserting below constriction of dart sac adjacent to each other; dart sac muscular, long, cylindrical, with medial constriction; presence of thin, short dart gland located inside distal portion of dart sac; dart sac ending at atrium level with vagina and penis; vas deferens surrounding dart sac medial constriction before entering penial complex; bursa copulatrix oval, with thin medium duct; free oviduct as long as bursa copulatrix duct; vagina shorter than penis; penial complex long, slender; flagellum thin, finger-like, ending level where vas deferens enters penial complex and epiphallus begins; epiphallus thin, widening at point of insertion of vas deferens; internally epiphallus with longitudinal, slender, undulating pleats; penis long, slender, twice or three times longer than epiphallus, entering at genital atrium level with dart sac and vagina; penial papillae absent; internal wall of penis with thin delicate longitudinal pleats, becoming taller with zigzag pattern in proximal penis zone; penial muscular band absent; penis sheath muscular, thick, overlapping all penis length, distally fused with atrium wall; atrium extremely short.

Habitat and Distribution (Fig. 20): *Epiphragmophora parodizi* is found in both Yungas and Pre-Puna biogeographic provinces, although live specimens have only been found in Yungas rainforest, mostly under trunks on the ground in contact with soil. It is distributed in Tucumán Province, northwestern Argentina.

Remarks: Specimens from northwestern Tucumán, in the locality of Gonzalo, have thin axial irregular, undulating lines on the shell surface of the first whorls and regular axial ribs separated by regular spaces on the the body whorl. These irregular thin lines are not present in the holotype and paratypes, but are constant in the specimens from Gonzalo, Tucumán.

Epiphragmophora proseni Hylton Scott, 1951 (Fig. 4A)

Epiphragmophora proseni Hylton Scott, 1951: 258, fig. 3.3.

Epiphragmophora proseni – Parodiz, 1957: 30.

Epiphragmophora proseni – Fernández & Rumi, 1984b: 243.

Holotype: MLP unnumbered, Argentina, Jujuy, Tumbaya Dept., Quebrada de Humahuaca, Tumbaya, II/1950, A. F. Prosen leg. ex Hylton Scott Collection.

Description:

Shell (Fig. 4A): Dextral, globose, solid; conic spire fairly elevated; medium size; with 4¼ convex whorls; dark brown to chestnut homogeneous color; peripheral band on body whorl absent; protoconch smooth; first spire whorls with axial ribs, body whorl malleated with axial to diagonal ribs, ventrally fine spiral lines well marked around umbilicus; suture well impressed; oval transversal aperture with peristome white, fine narrowly reflexed, interior of aperture chestnut brown; wall space between lips narrow without callus; body whorl descending abruptly behind aperture; umbilicus perspective, narrow, partly overlapped by peristomal lip.

Measurements: Holotype: D maj: 30.1 mm; D min: 24.4 mm; H: 15.3 mm; D ap: 17.7 mm; H ap: 15.0 mm.

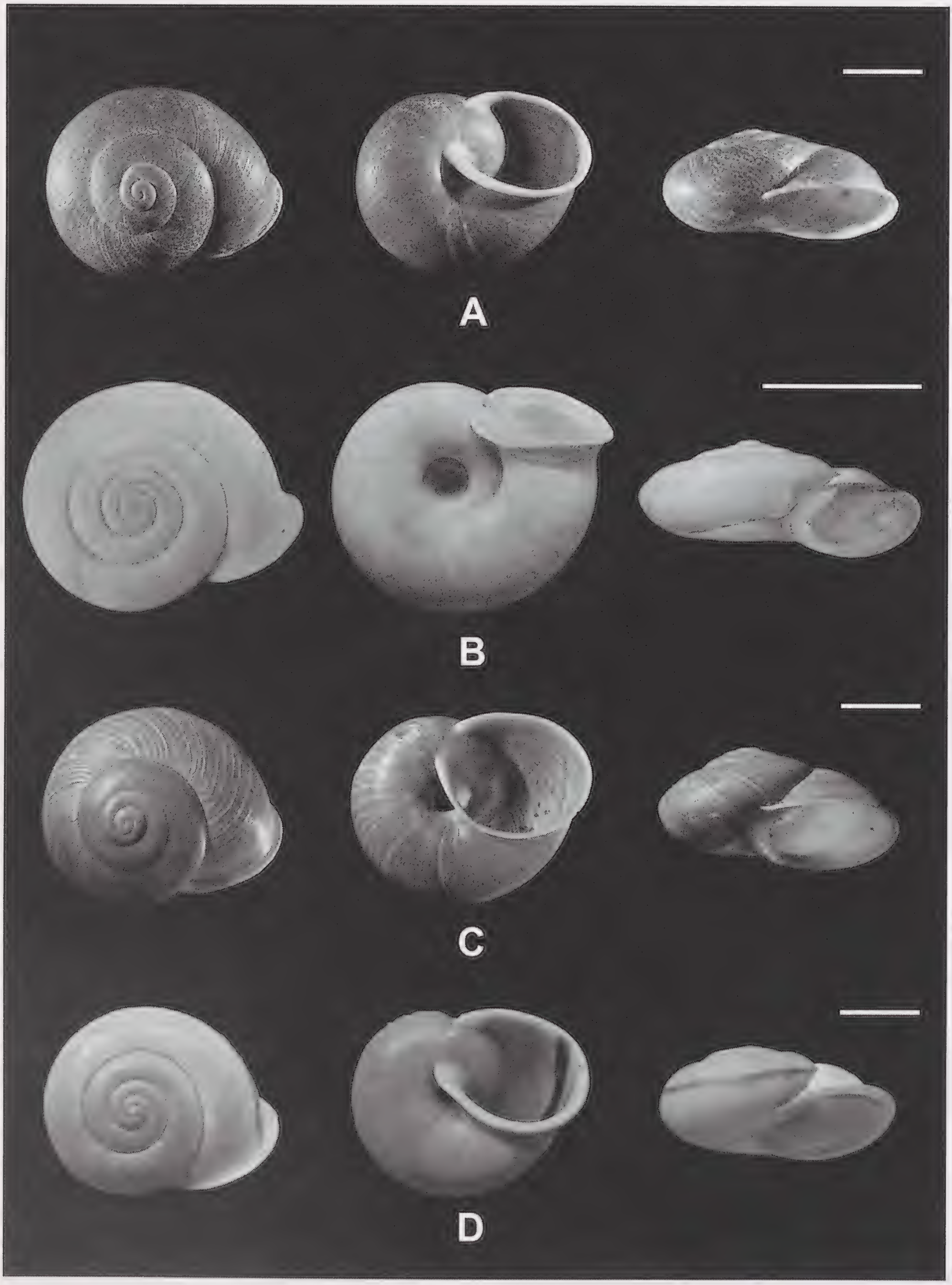


FIG. 4. Shells in dorsal, ventral and lateral view. A: *Epiphragmophora proseni* (MLP, holotype). Scale bar = 10 mm; B: *Epiphragmophora puella* (MLP, holotype). Scale bar = 20 mm; C: *Epiphragmophora puntana* (MACN 1284, lectotype). Scale bar = 10 mm; D: *Epiphragmophora rhathymos* (FML 14520). Scale bar = 10 mm.

Distribution: Single known locality of occurrence in Tumbaya, Jujuy Prov., Argentina.

Remarks: The original description of *E. proseni* was based on a single dry shell, the holotype, collected by A. Prosen. This shell is similar to *E. jujuyensis*, in most of its characters, except in the spire shape, which is shallower in *E. proseni* than in *E. jujuyensis* and the umbilicus, which is narrower in *E. proseni*. Hylton Scott stated that the shell color, the interior of the aperture chestnut brown and the absence of the peripheral band were the characters that allow the recognition of the present species. Nevertheless, all those mentioned characters are also present in some specimens of E. *jujuyensis*. The validity of the present species will remain dubious due to the unavailability of other specimens for comparison and the lack of anatomical studies. Neither dry shells nor live specimens of *E. proseni* were found in a field trip to the type locality.

Epiphragmophora puella Hylton Scott, 1951
(Figs. 4B, 21)

Epiphragmophora puella Hylton Scott, 1951: 253, fig. 3.1.
Epiphragmophora puella – Parodiz, 1957: 30.
Epiphragmophora puella – Fernández & Rumi, 1984b: 242.

Holotype: MLP unnumbered: Argentina, Catamarca, Capayán Dept., Quebrada de La Cébila.
Paratypes: MACN 16701, *Paratype*, Capayán Dept., Quebrada de La Cébila, 1950. MLP unnumbered, Paratypes, Capayán Dept., Quebrada de La Cébila.

Other Material Examined:
Argentina, Catamarca Prov.: FML 14378, Capayán Dept., Quebrada de La Cébila, 1,100 m, 15/IX/1968, leg. & det. by W. Weyrauch. MLP unnumbered, Capayán Dept., Quebrada de La Cébila, 13/III/1950. MLP unnumbered, Las Totoritas, Hylton Scott det., Martinez, A. leg.

Description:
Shell (Fig. 4B): Dextral, helicoidal, fragile; low to flat spire; small; 4¾ whorls equatorially subcarinated; pale yellowish to pale brownish; peripheral band absent; protoconch smooth; first whorl with axial growth lines, rest of teleoconch surface with tiny, oval pustules giving appearance of wrinkled surface; suture deeply impressed; aperture subcircular to subovoid; peristome whitish, thin, well expanded; body whorl not descending towards aperture or scarcely descending; umbilicus wide, perspective not overlapped by ventral columelar fold of peristome.
Measurements: Holotype: D maj: 18.0 mm; D min: 15.5 mm; H: 6.6 mm.
Paratypes (n = 5): D maj: 17.6–18.0 mm (17.6 mm); D min: 14.7–16.0 mm (15.3 mm); H: 6.4–8.6 mm (7.1 mm).

Distribution (Fig. 21): Southern Catamarca Prov., Argentina. Monte biogeographic province.

Remarks: This is a conspicuous species due to its small shell diameter, periphery of the body whorl and shell sculpture. The sculpture consisting of small regularly distributed pustules is unique among the species described in this paper. Fernandez & Rumi (1984b) described and illustrated the genitalia of *E. puella,* but unfortunately it was not possible to find the material on which the description was based. The published anatomical description is poor, and the connections and point of insertions of the different organs and their ducts are not clear in their figures.

Epiphragmophora puella is very difficult to find, usually deeply inside crevices of rocks, and it is only known from its type locality in Catamarca. It shares with *E. hieronymi* and *E. rhathymos* the same dry environment of the Monte biogeographic region.

Epiphragmophora puntana (Holmberg, 1909)
(Figs. 4C, 12A–C)

Helix puntana Holmberg 1909a: 9.
Epiphragmophora puntana – Paródiz, 1957: 29.
Epiphragmophora puntana – Fernández, 1973: 159.
Epiphragmophora puntana – Fernández & Rumi, 1984b: 243.

Lectotype: MACN 1284, Argentina, and San Luis, Cerro del Volcán.

Other Material Examined:

Material Dissected: Argentina, San Luis Prov.: FML 14518 A, Capital Dept., El Volcán, 22/I/2003, 33°12'26"S, 66°12'44"W, 1,200 m, M. G. Cuezzo & E. Dominguez leg.

Dry Material: Argentina, San Luis Prov.: FML 14393, Capital Dept., El Volcán, Mountain El Volcán, 33°15'02"S, 66°11'13"W, 910 m, 17/XI/2001. M. G. Cuezzo leg. & det. FML 14433, Potrero de Funes, 30 km from El Volcán, 33°12'02"S, 66°12'20"W, 1,000 m, 21/I/2003, M. G. Cuezzo & E. Dominguez leg. MLP 1665, Coronel Pringles Dept., Paso del Rey, Vigniatti leg. Argentina, Córdoba Prov.: FML 14519 (ex WW 1056), San Javier Dept., Las Cañadas. E. Palavecino leg., 1939, det. by J. J. Parodiz. MLP unnumbered, Punilla Dept., Pampa de Olain. Cicheros, J. leg., I/1967, ex collection Hylton Scott. MLP unnumbered, Candelaria de la Sierra, Cichero-Martinez leg. XII/1966, ex collection Hylton Scott.

Description:

External Features: Body color reddish to dark orange, ommatophores with darker red to brown color; double row of pustules regularly located on middle dorsal cephalic region running from mantle to space between ommatophores well marked.

Shell (Fig. 4C): Dextral. helicoidal, globose, semisolid; medium size; spire conic, elevated; medium to large; 4½ convex whorls; yellowish to golden brown; single, thin dark brown supraperipheral band present in body whorl; protoconch smooth; axial whitish to light yellow ribs regularly distributed in all whorls except in protoconch, ribs more evident in body whorl; space between ribs with fine line growth crossed by other fine transversal lines slightly marked; suture well impressed; aperture transverse oval, internal wall of aperture light chestnut; peristome thin, slightly expanded, whitish; thin parietal callus usually raised; body whorl strongly descending behind aperture; umbilicus perspective, partly overlapped by peristomal lip.

Measurements: Lectotype: D maj: 30.7 mm; D min: 27.0 mm; H: 16.6 mm; D ap: 18.5 mm. Specimens from Type Locality (n = 11): D maj: 30.0–26.3 mm (28.7 mm); D min: 26.7–22.9 mm (25.6 mm); H: 17.8–15.0 mm (15.9 mm); D ap: 17.8–15.9 mm (17.1 mm); H ap: 15.7–13.6 mm (14.9 mm).

Jaw: corneous, arcuate, orange, thin; four central ribs, middle ones wider than both laterals.

Pallial Complex: Kidney extending about 60% of pulmonary roof length; pulmonary roof homogeneous pale gray, densely traversed by net of thin veins especially close to mantle collar.

Genitalia (Fig. 12A–C): Terminal genitalia with dart sac complex consisting of single dart sac and two mucous glands; both glands round shaped, left slightly bigger than right, both distally fused with atrium wall; each mucous gland with thin efferent duct distally fused with each other into single duct inserting below constriction of dart sac; dart sac long, muscular, cylindrical, with medial constriction; dart papillae thick, cylindrical, as long as distal portion of dart sac; distal portion of dart sac below constriction shorter than proximal; dart sac ending at atrium level with vagina and penial complex; bursa copulatrix sac round, with thin medium length duct basally wider; sac and duct of bursa copulatrix neatly delimited; free oviduct thin, longer than vagina; vagina shorter than penis; penial complex short to medium; flagellum finger-like with thick distal portion ending level where vas deferens enters penial complex and epiphallus begins; epiphallus shorter than penis, externally differentiated from it due to its smaller diameter; penial retractor muscle thin, long, inserting in median portion of epiphallus; penis cylindrical, broader at proximal portion, thinner at distal; penis longer than epiphallus, entering at genital atrium level with dart sac and vagina; internal sculpture of penis well differentiated into two regions, smooth strength pleats located in proximal portion of penis, externally overlapped by penis sheath and thick zigzag folds in distal portion of penis, externally free of penial sheath; penial papillae short, rectangular, with surface sculpture consisting of vertical bands; penis sheath muscular, distally fused with atrium wall.

Habitat: *Epiphragmophora puntana* inhabits mountains with xerophilic vegetation and is found always associated to rocks, usually inside narrow crevices formed between big rocks. This species inhabit the Monte biogeographic province.

Distribution: *Epiphragmophora puntana* is known to live in central and northeastern San Luis Province, Argentina, and occasionally have been found in southern Córdoba Province.

Epiphragmophora quirogai Cuezzo, n. sp. (Figs. 7B, 18D–F, 21)

Holotype: FML 14721A, Argentina, La Rioja, Sierra de Velazco, Anillaco, 1/III/1998, G. Perotti leg.

Paratypes: FML 14719, Argentina, La Rioja, Sierra de Velazco, close to Anillaco, 2/III/1998, Perotti, M.G. leg. FML 14429 A, type locality, F. Cruz leg. MLP unnumbered, type locality.

Other Material Examined:

Dissected Material: Argentina, La Rioja Prov.: FML 14429A, Sierra de Velazco, close to Anillaco, II/1998, Cruz, F. leg.; FML 14428A , Sierra de Velazco, 1996, F. Cruz, leg.

Dry Material: Argentina, La Rioja: FML 14722, Castro Barros Dept., Santa Vera Cruz, 1/IV/1998, M. G. Cuezzo & E. Dominguez leg. Argentina, Catamarca: FML 447, Belén Dept. Belén, 25/I/1960, leg. Willink. FML 14369, Cuesta de La Puerta, 900 m, close to Pirquitas, W. Weyrauch leg. & det. FML 152, Andalgalá, 19/I/1934, Schreiter leg. FML 14724, Tinogasta Dept., Cuesta de Zapata, 27°49'00"S, 67°18'52"W, 1,710 m, 28/XI/2003, M. G. Cuezzo & C. Ituarte leg.

Diagnosis: Shell bright cream to bright pink, without peripheral pigmented band in body whorl; shell subglobose; spire and body whorl convex; spire shallow; suture deeply impressed; terminal genitalia with one mucous gland sac-like incompletely surrounding basal dart sac and vagina, the other oval-elongate; both mucous glands with efferent ducts that fuse together before inserting below constriction of dart sac; epiphallus double in length respect to penis.

Etymology: The species is named after Juan Facundo Quiroga, also known as "Tigre de Los Llanos", who fought for the independence of the Agentinean provinces towards a national organization and later was misjudged by history.

Description:

External Features: Animal with even black color of its body; single dorsal row of pustules in cephalic region running from mantle collar to ommatophores.

Shell (Fig. 7B): Dextral, helicoidal, depressed, semisolid; spire low, conical; small to medium size; 4¼ convex whorls; bright cream to bright pink; peripheral pigmented band absent; protoconch smooth; teleoconch surface with thin axial growth lines; body whorl surface with thin axial growth lines crossed by irregular peripheral lines, giving malleated appearance in some specimens; suture deeply impressed; aperture subcircular, with straight basal lip; peristome whitish, thin, slightly reflexed; parietal wall space between margins of upper and basal lip narrow; body whorl slightly descending towards aperture; umbilicus wide, not overlapped by ventral columelar fold of peristome.

Measurements: Holotype: DM: 20.4 mm; D min = 17.7 mm; H = 8.4 mm; D ap = 10.2 mm; H ap = 8.8 mm.

Paratypes FML 14719 (n = 10): DM: 19.4–21.6 mm (18.5 mm); D min: 15.8–18.8 mm (17.5 mm); H: 8.3–10.0 mm (9.0 mm); D ap: 10.0–12.0 mm (10.9 mm); H ap: 8.0–10.2 mm (9.2 mm).

Jaw: Arched, thin, orange, with five central thin ribs; ribs very pronounced and delimited; borders of ribs projecting at both upper and lower edges.

Pallial Complex: Lung roof without dark spots, homogeneously pale cream; kidney extending 50% of total pulmonary roof length; main pulmonary vein spliting proximally in two mayor veins that distally are divided into several minor veins before reaching mantle collar; portion of lung roof between rectum and kidney crossed by several minor transversal ramified veins.

Genitalia (Fig. 18D–F): Dart sac complex in terminal genitalia consisting of single dart sac and two unequal mucous glands; right mucous gland sac-like, incompletely surrounding distal zone of vagina and dart sac, distally fused with atrial wall; left mucous gland oval-elongate, opening into thick efferent duct; left gland duct fusing with right gland thinner duct into single efferent duct inserting in medial zone of dart sac below its constriction; dart sac muscular, long, cylindrical, with medial constriction; thin, internal dart gland located in distal portion of dart sac; dart sac ending at atrium level with vagina and penis; bursa copulatrix round, with thick short duct; vagina shorter than penis; penial complex long; flagellum thin, fingerlike, less than half epiphallus length ending level where vas deferens enters penial complex and epiphallus begins; internal wall proximal epiphallus sculpture with thick pilaster beginning at point of insertion of vas deferens and progressively tapering towards distal epiphallus; epiphallus proximal portion thick, progressively becoming thinner, epiphallus double penis total length; penial retractor inserting in medial portion of epiphallus; vas deferens forming loop around penial retractor close to vas deferens point of insertion; penis cylindrical entering genital atrium level with dart sac and vagina; penial papillae short, triangular; internal wall of penis with thin delicate longitudinal pleats; penis sheath short, muscular, overlapping terminal portion of penis; atrium usually long.

Habitat and Distribution (Fig. 21): ***Epiphragmophora quirogai*** is found under rocks in dry environments of Monte biogeographic regions in Catamarca and La Rioja provinces, Argentina.

Remarks: ***Epiphragmophora quirogai*** is one of the smallest species of the genus. Some specimens in museums were originally identified as *E. hieronymi*, as its general shape is similar and their areas of distribution partially overlapped. However, identification of both species is possible by different shell and genitalia characters. A comparison of some characters among the closest species – *E. hieronymi, E. villavilensis, E. puella* and ***E. quirogai*** – is presented in Table 1.

Epiphragmophora rhathymos (Holmberg, 1912)
(Figs. 4D, 12D–E, 20)

Helix (*Eurycampta*) *rhathymos* Holmberg, 1912: 20.
Helix (*Eurycampta*) *monographa* – Doering, 1874: 450, *non Helix Eurycampta monographa,* Burmeister, 1861: 77.
Epiphragmophora trenquelleonis rhathymos – Parodiz, 1957: 29.
Epiphragmophora trenquelleonis rhathymos – Fernández, 1973: 161.
Epiphragmophora rhathymus – Fenández & Rumi, 1984b: 244.

Lectotype: SMF 20397, Argentina, Quebrada del Tola, Sierra de Catamarca, Hieronymus leg., Doering, det. (Zilch, 1971)
Paralectotype: MACN 1289, Argentina, Catamarca, El Desmonte. Holmberg Collection.

Other Material Examined:
Dissected Material: Argentina, Catamarca Prov.: FML 14520A, Esquiú Dept., Las Pirquitas, 28°16’07"S, 65°44’W, 760 m, 13/XI/2001, M. G. Cuezzo leg. & det. Argentina, Tucumán Prov.: FML 14404A, J. B. Alberdi Dept., Escaba. XI/1996, M. G. Cuezzo leg. Argentina, Córdoba Prov.: FML 14440 A, Cruz del Eje Dept., route 15 between Villa de Soto and La Higuera, 30°59’12"S, 65°04’45"W, 650 m, 14/XI/2001, M. G. Cuezzo leg. & det.
Dry Material: Argentina, Salta Prov.: FML 125, La Candelaria Mountain, 15/VII/1931, Schreiter, R. leg., W. Weyrauch det. FML 14526, Metán Dept., on road to Cabra Corral, 25°16’34"S, 65°18’45"W, M. G. Cuezzo leg. MLP unnumbered, La Viña Dept., Alemania, Birabén leg., ex Hylton Scott collection. FML 14734, Cafayate, Yacochuya del Norte, 26°02’14"S, 66°01’34"W, 2,000 m, 25/III/2005, M. G. Cuezzo leg. & det. Argentina, Catamarca Prov.: FML 14372, Esquiú Dept., Dique Las Pirquitas, 25 km N from Catamarca, 850 m, 21/I/1968, W. Weyrauch leg. & det. FML 563, El Rodeo, 1/I/1958, R. Golbach leg., J. J. Parodiz det. FML 14521, Capayán Dept., Quebrada La Cébila, 1,100 m, 15/XI/1968, W. Weyrauch leg. & det. FML 14525, El Desmonte, km 1331 on road from Catamarca to Pirquitas, 700 m, 15/XI/1968, W. Weyrauch leg. & det. FML 14523, Esquiú Dept., El Hueco, 7 km from Catamarca, on road to Pirquitas, 600 m, 15/IX/1968, leg. & det. by W. Weyrauch. FML 14522, Ambatos Dept., Cuesta de la Puerta, between Pirquitas and Ambato, 900 m, 15/IX/1968, leg. & det. by W. Weyrauch. FML 14450, Ambatos Dept., on road from Las Estancias to Singuil, 27°36’20"S, 65°58’36"W, 1,920 m, 29/XI/2003, M. G. Cuezzo leg. FML 14722, Ambatos Dept. Las Chacritas, on road to Singuil from Las Estancias, 27°41’42"S, 65°55’09"W, 1,780 m, 30/XI/2003, M. G. Cuezzo leg. FML 538, Close to El Alamito, km 80 on road from Andalgalá to Tucumán, 1,600 m, Weyrauch, Willink & Tomsic leg. J. J. Parodiz det.

Description:
External Features: Body dark red with dark brownish to blackish background.
Shell (Fig. 4D): Dextral, subglobose, semisolid; spire shallow conical; medium to large size; 4½ convex whorls; pale brown to pale chestnut; body whorl with one equatorial brown peripheral band that also lines the suture but is absent on spire; protoconch smooth; surface of body whorl with very thin growth-lines and peripheral lines, giving appearance of a malleated surface; suture shallow; aperture

circular with white to chestnut, thick, reflexed peristome; parietal wall space between margins of upper and basal lip narrow; body whorl abruptly descending behind aperture; umbilicus deep, narrow, partly overlapped by basal columelar peristomal lip.

Measurements: Paralectotype MACN 1289: D maj: 30.2 mm; D min: 25.4 mm; H: 15.6 mm; D ap: 18.1 mm.

Specimens from Quebrada de La Cébila, Catamarca (n = 10): DM: 30.8–27.7 mm (29.0 mm); D min: 26.8–23.6 mm (25.0 mm); H: 16.6–14.9 mm (15.5 mm); D ap: 18.8–17.3 mm (18.0 mm); H ap: 16.6–14.0 mm (15.3 mm).

Jaw: Arched, thin, orange, with three to six central wide ribs; ribs not very pronounced or delimited; borders of ribs projecting at both edges.

Pallial Complex: Lung roof without dark spots; kidney extending about 60% of pulmonary roof length; main pulmonary vein splitting into two mayor veins in distal portion of pulmonary roof.

Genitalia (Fig. 12D, E): Dart sac complex in terminal genitalia consisting of single dart sac with two mucous glands; right mucous gland sac-like, distally fused with atrium wall, opening into a thin duct; left mucous gland oval-elongate, smaller than dart sac, opening into thin distal duct; both mucous gland efferent ducts distally fused with each other before entering dart sac; dart sac muscular, thick, cylindrical, with medial constriction below which mucous glands duct inserts; dart papillae cylindrical, half as long as distal portion of dart sac; dart sac ending at atrium level with vagina and penial complex; vas deferens looping around dart sac constriction before running towards penial complex; bursa copulatrix sac oval with thick, short to medium duct; sac and duct of bursa copulatrix not neatly delimited; vagina short, as long as penis; penial complex medium in length; flagellum thin, cylindrical, with thick distal portion ending level where vas deferens enters penial complex and epiphallus begins; epiphallus thin, as long as penis; penial retractor muscle inserting in median portion of epiphallus; penis cylindrical, thicker than epiphallus, entering at genital atrium level with dart sac and vagina; penial papillae absent; internal wall of penis with thin undulating pleats; penis sheath simple, muscular, overlapping distal portion of penis and distally fused with atrium wall; penial muscular band absent; atrium very short.

Habitat: Usually found under rocks or adhered to walls in narrow rock crevices. In Catamarca, *E. rhathymos* inhabits xerophilic environments where also some bulimulids and odontostomids species are abundant. Its vertical habitat distribution ranges between 600 and 2,000 m above sea level.

Distribution (Fig. 20): *Epiphragmophora rhathymos* is more abundant in Catamarca Province, but it is also found in some localities of Salta, Tucumán and Córdoba provinces, northwestern and central Argentina. Although is distributed mainly in the Chaco biogeographic province, there are some localities in Salta and Tucumán, where the vegetation is a transition to Yungas rainforest.

Remarks: *Epiphragmophora rhathymos* is similar to *E. trenquelleonis* in general shell morphology except for the malleations sculpture on the body whorl. Shell color is also very different. Body color is similar but usually animals of *E. rhathymos* tend to be more reddish.

Epiphragmophora saltana Ancey, 1897
(Figs. 5A, 13A–C, 19)

Epiphragmophora saltana Ancey, 1897: 9.
Epiphragmophora saltana – Parodiz, 1957, 71: 30.
Epiphragmophora saltana – Fernández & Rumi, 1984b: 246.

Holotype: BMNH, Salta Prov., Argentina.

Other material Examined:

Dissected Material: Argentina, Salta Prov.: FML 14392A, Candelaria Dept., El Tala, X/1998, E. Dominguez leg. FML 14391A, Candelaria Dept., Route 6, 6 km from El Jardin, close to El Tala river, XI/1996, M. G. Cuezzo & C. Ituarte leg.

Dry Material: Argentina, Salta Prov.: FML14391, Candelaria Dept., Route 6, 6 km from El Jardin, close to El Tala river, XI/1996, M. G. Cuezzo & C. Ituarte leg. MACN 20369: Rosario de la frontera.

Description:

External Features: Animal body yellowish to light brown with double row of dorsal pustules in cephalic region from mantle collar to the ommatophores.

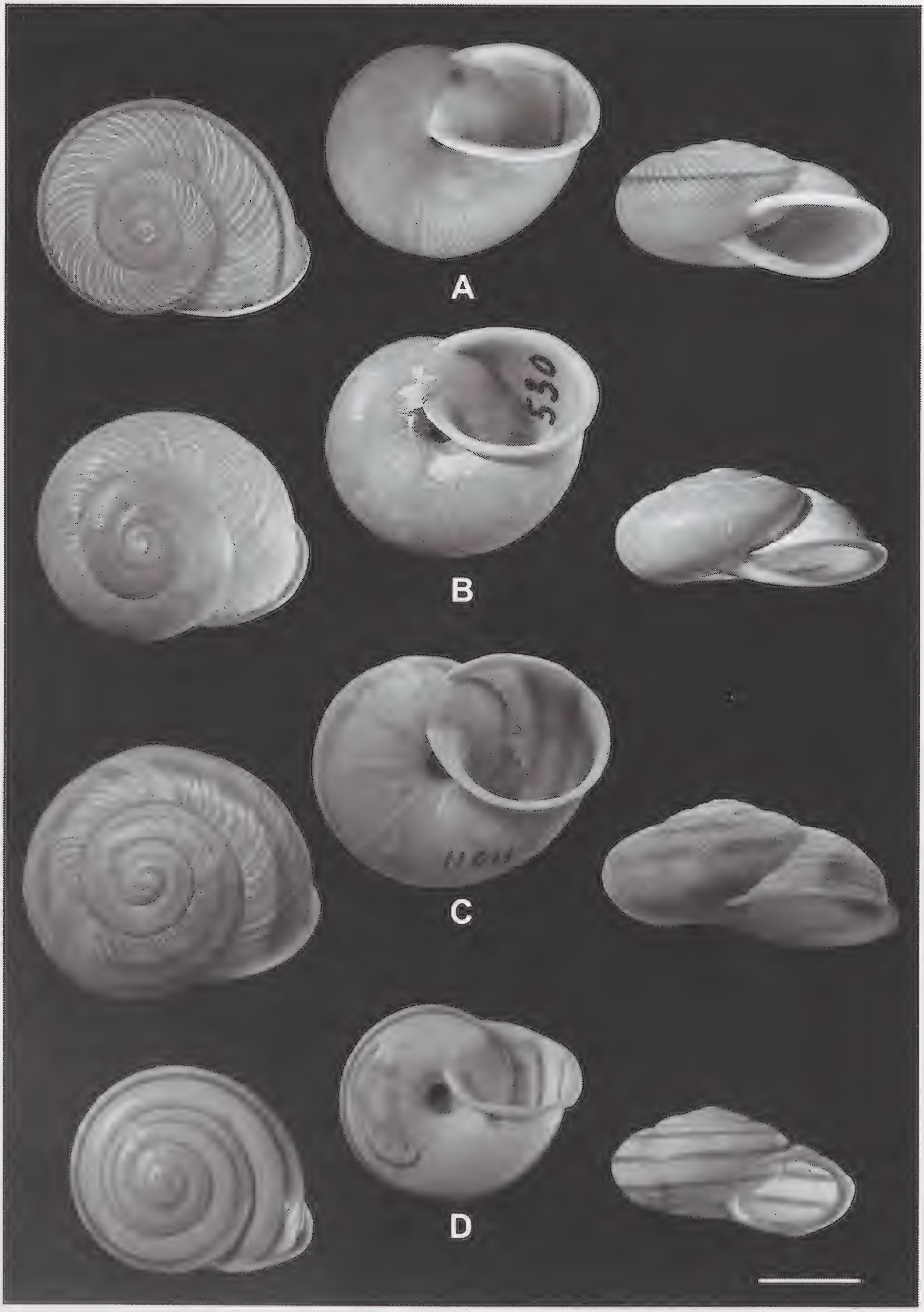


FIG. 5. Shells in dorsal, ventral and lateral view. A: *Epiphragmophora saltana* (NHM, holotype); B: *Epiphragmophora tomsici* (FML 530, holotype); C: *Epiphragmophora trifasciata* (MACN 24406, holotype); D: *Epiphragmophora trigrammephora* (MNHN, syntype). Scale bar = 15 mm.

Shell (Fig. 5A) Dextral, helicoidal, depressed-globose, solid; spire shallow conic; medium size; 4½ convex whorls; yellowish to golden; thin pigmented dark brown spiral band on body whorl; protoconch smooth; axial pale ribs regularly distributed in all whorls, surface between ribs strongly malleated; suture well impressed; aperture transverse, oval; peristome white, thick, reflexed; parietal callus usually raised, whitish, thin; body whorl abruptly descending towards aperture; umbilicus narrow partly to totally overlapped by peristomal lip.

Measurements: Ancey's original description (two shells): D maj: 32 mm, 29 mm; D min: 25½ mm, 25.0 mm; H: 17½ mm, 16½ mm.

Specimens from Type Locality Salta, El Tala (n = 16): D maj: 23.8–29.3 mm (27.2 mm); D min: 21.8–25.6 mm (23.6 mm); H: 13.4–16.4 mm (14.8 mm).

Specimens from Salta, El Jardín (n = 4): D maj: 26.6–29.1 mm (28.0 mm); D min: 22.0–24.6 mm (23.6 mm); H: 14.2–17.4 mm (15.2 mm).

Jaw: Arched, thin with five to seven thin ribs; margins of ribs projecting at both edges.

Pallial Complex: Lung roof without dark spots; kidney extending 60% of total pulmonary roof length; main pulmonary vein subdivided in two mayor veins proximally, distally they further divide into several minor veins before reaching mantle collar.

Genitalia (Fig. 13A–C): Dart sac complex in terminal genitalia consisting of single dart sac and two mucous glands; right gland sac-like, enveloping distal zone of vagina and dart sac, not fused to atrial wall; left mucous gland oval-elongate, opening into efferent duct that joins and fuses with thinner right gland efferent duct forming unique duct; dart sac muscular, cylindrical, with medial constriction, below which mucous glands duct inserts; thin dart gland, located inside distal portion of dart sac; dart sac gland half length of distal dart sac portion; dart sac ending at atrium level with vagina and penial complex; bursa copulatrix round to oval with thick, short to medium length duct; vagina shorter than penis; penial complex long; flagellum thin, finger-like, short, gradually tapering towards end; flagellum ending level where vas deferens enters penial complex and epiphallus begins; epiphallus long, thinner than penis, not externally differentiated from it; penis thin, slender, entering genital atrium level with dart sac and vagina; penial papillae absent; internal wall of penis with thin undulating parallel pleats; penis sheath muscular, thick, distally multilayered, fused with atrium wall, proximal penis sheath thin, single layer, overlapping all penis length; penial muscular band absent; atrium extremely short.

Habitat: Subtropical humid rainforest in Yungas biogeographic province, northwestern Argentina. Live specimens usually found below humid tree trunks on the floor in contact with soil.

Distribution (Fig. 19): *Epiphragmophora saltana* is known only from southern Salta Province, northwestern Argentina.

Remarks: *Epiphragmophora saltana* is similar in shell morphology to *E. birabeni.* It shares shell characters such as the presence of axial regular ribs with *E. birabeni* and *E. costellata*. The anatomy of these two species is unknown.

Epiphragmophora tomsici Fernández & Rumi, 1984
(Figs. 5B, 13D, E)

Epiphragmophora tomsici Fernández & Rumi, 1984b: 246, pl. 7, figs. 6–8.

Holotype: FML 530, Argentina, Catamarca, Dept. Andalgalá, 20 km from Andalgalá, road to Cafayate, 1,800 m, 26/II/1962, W. Weyrauch Col.

Other Material Examined:

Dissected Material: Argentina, Catamarca Prov.: FML 14529 A, Andalgalá Dept., junction of Blanco river with Candado river where Andalgalá river begins, 27°27'23"S, 66°16'15"W, 25/V/1997, Schrocchi, G. leg. FML 14445 A*, Andalgalá Dept., 20 km from Andalgalá on road to Mina Capillitas, 27°29'07"S, 66°22'57"W, 1,800 m, 29/XI/2003, Ituarte, C. leg. Argentina, Tucumán Prov.: FML 14390A, Trancas Dept., road 364 close to Rearte, 26°22'53"S, 65°31'35"W, 1,130 m, 15/X/1996, M. G. Cuezzo leg. & det. FML 14527A, Trancas Dept., road 364 close to Rearte, 26°22'53"S, 65°31'35"W, 1,130 m, 30/III/1997, M. G. Cuezzo leg. & det. FML 14528 A, Trancas Dept., on road 364, close to Rearte, 26°22'53"S, 65°31'35"W, 1,130 m, 7/5/2002, M. G. Cuezzo leg.

Dry Material: Argentina, Catamarca Prov.: FML 530, Paratypes, Andalgalá Dept., 20 km from Andalgalá on road to Santa María, 1,800 m, 8/XII/1968, W. Weyrauch leg. & det. FML 14725, Andalgalá Dept., Cuesta Mina Capillitas, 27°29'54"S, 66°22'53"W, 1,630 m, 29/XI/2003, M. G. Cuezzo & C. Ituarte leg. Argentina, Tucumán Prov.: FML 14390, Trancas Dept., road 364 close to Rearte, 26°22'53"S, 65°31'35"W, 1,130 m, 15/X/1996. M. G. Cuezzo leg. & det. FML 795, Trancas Dept., 3 km S of San Pedro de Colalao, 1,200 m, J. S. Guanuco leg., W. Weyrauch det. FML 1210, Trancas Dept., 6 km S of San Pedro de Colalao, 1/XI/1963, Guanuco, J. S. leg. FML 14288, Trancas Dept., San Pedro de Colalao, 1/II/1994, Dragh, F. leg. FML 11313, Trancas Dept., Vipos river, 10 km from Vipos gas station, 1/V/1964, R. Herbst leg.

Description:

External Features: Animal variable in body color, specimens from Tucumán Province usually homogeneously light brown; specimens from type locality in Catamarca Province bright red to bright orange; shell color similar in both localities.

Shell (Fig. 5B): Dextral, helicoidal, globose, solid; spire shallow conic, with thin axial growth lines; medium size with 4½ convex whorls; light brown to light chestnut; peripheral band absent; protoconch smooth; axial whitish ribs regularly distributed on body whorl; surface between ribs in body whorl strongly malleated, forming distinctive white reticulated pattern; suture well impressed; aperture transverse oval; peristome white, thick, reflexed; parietal callus usually raised, whitish, thin; parietal space between lips narrow; body whorl abruptly descending towards aperture; umbilicus open partly overlapped by peristomal lip.

Measurements: Holotype: D maj: 27.2 mm; D min 24.0 mm; H: 13.8 mm.

Paratypes (n = 4): D maj 24.0–26.3 mm (25.3 mm); D min: 21.6–24.9 mm (22.7 mm); H: 12.0–15.2 mm (13.7 mm).

Other Material: FML, Tucumán, Los Rearte (n = 10): D maj: 25.0–28.8 mm (27.3 mm); D min: 22.4–25.0 mm (23.6 mm); H: 14.0–15.3 mm (14.6 mm).

Jaw: Arched with five thin central ribs; margins projecting at both edges.

Pallial Complex: Lung roof homogeneously pale cream without pigmented dark spots, lung roof heavily traversed by minor thin venation, especially between kidney and rectum; kidney length 60% of total lung roof length; main pulmonary vein bifurcated into two thick veins in distal lung until mantle collar.

Genitalia (Fig. 13D, E): Dart sac complex in terminal genitalia consisting of single dart sac and two mucous glands; right gland sac-like, enveloping distal portion of vagina, dart sac and atrium in all its length, not fused with the atrial wall; left mucous gland cylindrical ending at thin efferent duct that joins with thinner duct of right gland closed to insertion point in dart sac; dart sac muscular, cylindrical, with medial constriction below which mucous glands duct inserts; thin, cylindrical dart gland located inside distal portion of dart sac; dart papillae as long as distal portion of dart sac; dart sac ending at atrium level with vagina; bursa copulatrix sac oval with thick medium length duct; limit between sac and duct not sharp; vagina medium length but shorter than penis; penial complex long; flagellum thin, long, ending level with where vas deferens enters penial complex and epiphallus begins; proximal epiphallus widening at point of insertion of vas deferens; epiphallus as long as penis, not differentiated from it externally; penial retractor muscle inserting in distal portion of epiphallus; penis simple entering at genital atrium level with dart sac and vagina; penial papillae absent; internal wall of penis with thin undulating folds in all its length; penis sheath muscular but thin, basally multilayer, overlapping half penis length, distally fused with atrium wall; penial muscular band absent; atrium short, totally overlapped by mucous gland.

Habitat: *Epiphragmophora tomsici* is found in xerophilic environments, usually hidden inside rock crevices or under fallen cactus.

Distribution: This species is found in Catamarca and Tucumán provinces, northwestern Argentina, in the Yungas and Chaco biogeographic provinces.

Remarks: Genitalia similar to *E. saltana* except for the length of the bursa copulatrix duct, shape of bursa copulatrix sac, length of atrium and length of flagellum. Shells of both species are very different in size, color and sculpture of the body whorl.

Epiphragmophora trenquelleonis (Grateloup, 1851)

(Figs. 6B, 14A–C, 21)

Helix trenquelleonis Grateloup, in Pfeiffer, 1851: 13, pl. 3, fig. 3–5.

Helix group *Eurycampta monographa* – Burmeister, 1861: 77, *non Helix Eurycampta monographa* Doering, 1874, = *E. rhathymus* (Holmberg).

Helix group *Eurycampta hidalgonis* Doering, 1875a: 315.

Helix group *Eurycampta Trenquelleonis* – Doering, 1875b: 156.

Helix hidalgonis – Kobelt, 1878: 144.

Helix doeringiana – Holmberg, 1909a: 9.

Epiphragmophora trenquelleonis – Parodiz, 1957: 29.

Epiphragmophora (*Doeringia*) *trenquelleonis* – Zilch, 1959–1960: 656.

Epiphragmophora trenquelleonis hidalgonis – Hylton Scott, 1965: 24.

Epiphragmophora trenquelleonis – Fernández & Rumi, 1984b: 247.

Lectotype SMF 7708, Argentinean: Vorberge der Sierra de Cordoba, namentlich die Sierra Chica und die Sierra de Pocho. A. Doering.

Other Material Examined:

Dissected Material: Argentina, Cordoba Prov.: FML 14434A, San Justo Dept., San Esteban, 5/XII/1986, leg. Z. Tomsic. FML 14435A Santa Maria Dept., 20 km S Alta Gracia, 28/I/1998, E. Dominguez leg. FML 14436A Calamuchita Dept., Rio Tercero, 28/I/1998, M. G. Cuezzo leg. FML 14437A, Punilla Dept., Valle Hermoso, La Falda, 28/I/1998, leg. M. G. Cuezzo FML 14438A, Cruz del Eje Dept., Route 15 between Villa de Soto and La Higuera, 30°59’12"S, 65°04’W, 650 m, 14/XI/2001, M. G. Cuezzo leg. & det. MLP unnumbered: Embalse Rio Tercero. Ringuelet leg., 1946. MLP unnumbered: Cabana.

Argentina, San Luis Prov.: FML 1314, Ayacucho Dept., 20 km from Santa Rosa, on road to Quines, 650 m, 23/II/1968, leg. W. Weyrauch. FML 14439 A, San Martín Dept., route 2 between Libertador General San Martín and Quines, 32°20’26"S, 65°41’W, 815 m, 15/XI/2001, leg. & det. Cuezzo, M. G.; FML 14768 A, San Martín Dept., close to Quines, 32°17’19"S, 65°41’21"W, 555 m, 16/XI/2001, leg. & det. M. G. Cuezzo.

Dry Material: Argentina, Córdoba Prov.: FML 14717, Rio Seco Dept., 5 km North of Villa María, 440 m, 25/XII/1967, W. Weyrauch leg. FML 10889, Rio Seco Dept., 5 km N of Villa María, 440 m, 25/XII/1967, Weyrauch leg & det. MLP 446, Rio Seco Dept., El Zapallar, Borello, A. leg. 24/V/1964, Hylton Scott det. FML 792, Ischilín Dept., 7–8 km from Charbonier, on road to Copacabana, 920 m, 26/III/1967, leg. & det. W. Weyrauch. MLP 1658-1, Ischilín Dept., Quilinos, Biraben, M., 5/III/1945. FML 705, Punilla Dept., close to Villa Carlos Paz, 700 m, 23/III/1967, leg. & det. W. Weyrauch. FML 929, Punilla Dept., Punilla Valley, San Esteban, leg. & det. W. Weyrauch. FML 14362, Punilla Dept., Villa Carlos Paz, 700 m, 23/III/1967, Weyrauch leg & det. FML 14361, Punilla Dept., 6 km from Capilla del Monte, 900 m, 25/XII/1967, Weyrauch leg. MLP 2164, Punilla Dept., Capilla del Monte. FML 910, Pocho Dept., Sierra de Pocho between Las Palmas and Chancani, 1,050 m, 12/XI/1967, leg. & det. W. Weyrauch. FML 14289, Pocho Dept., NW of Sierra de Pocho close to Chancani, 900 m FML 225, Colon Dept., Cabana, Sierra Chica, leg. & det. by Hylton Scott. FML 12766, Colón Dept., Sierra Chica, leg. & det. Hylton Scott. MLP 995, Colon Dept., Rumi Huasi, Cabana. MLP 4248-1, Colón Dept., Unquillo, Cabana, ex Hylton Scott collection. MLP 1640, Colón Dept., Rio Ceballos, 3/II/1939. FML 1319, Santa María Dept., Villa Anisacate, 40 km, SE of Córdoba, 21/III/1968, leg. & det. W. Weyrauch. MLP 4247, Santa María Dept, Alta Gracia, Hylton Scott leg. FML 716, Dean Funes Dept., San Vicente Mountain, 8 km E of Dean Funes, 300 m, 26/III/1967, Weyrauch leg. & det. FML 14375, Dean Funes Dept., San Vicente Mountain, 8 km E of Dean Funes, 800 m, 6/IV/1969, Weyrauch leg. FML 14363, San Alberto Dept., Los Pozos, on road from Mina Clavero to Villa Dolores, 21/II/1968, leg. & det. by Weyrauch. MLP 4249, Totoral Dept., Las Cortaderas, Hylton Scott-Biraben leg. 1949. MLP 4288, Calamuchita Dept., Las Bajadas, Ringuelet, R. leg. 06/I/1943. MLP unnumbered, Cuesta de Chancani, Cichero-Martinez leg., XI/1966. MLP 4306, Calamuchita Dept., San Agustín, X/1970. MLP 1674, La Calera, Pacheco, A. leg., 1934. Argentina, San Luis Prov.: FML 1314, Ayacucho Dept., on road to Quines, 20 km from Santa Rosa, 650 m, 23/II/1968, W. Weyrauch leg. MLP 4309, Piedra Blanca, II/1949. MLP 981, Ayacucho Dept., Quines, El

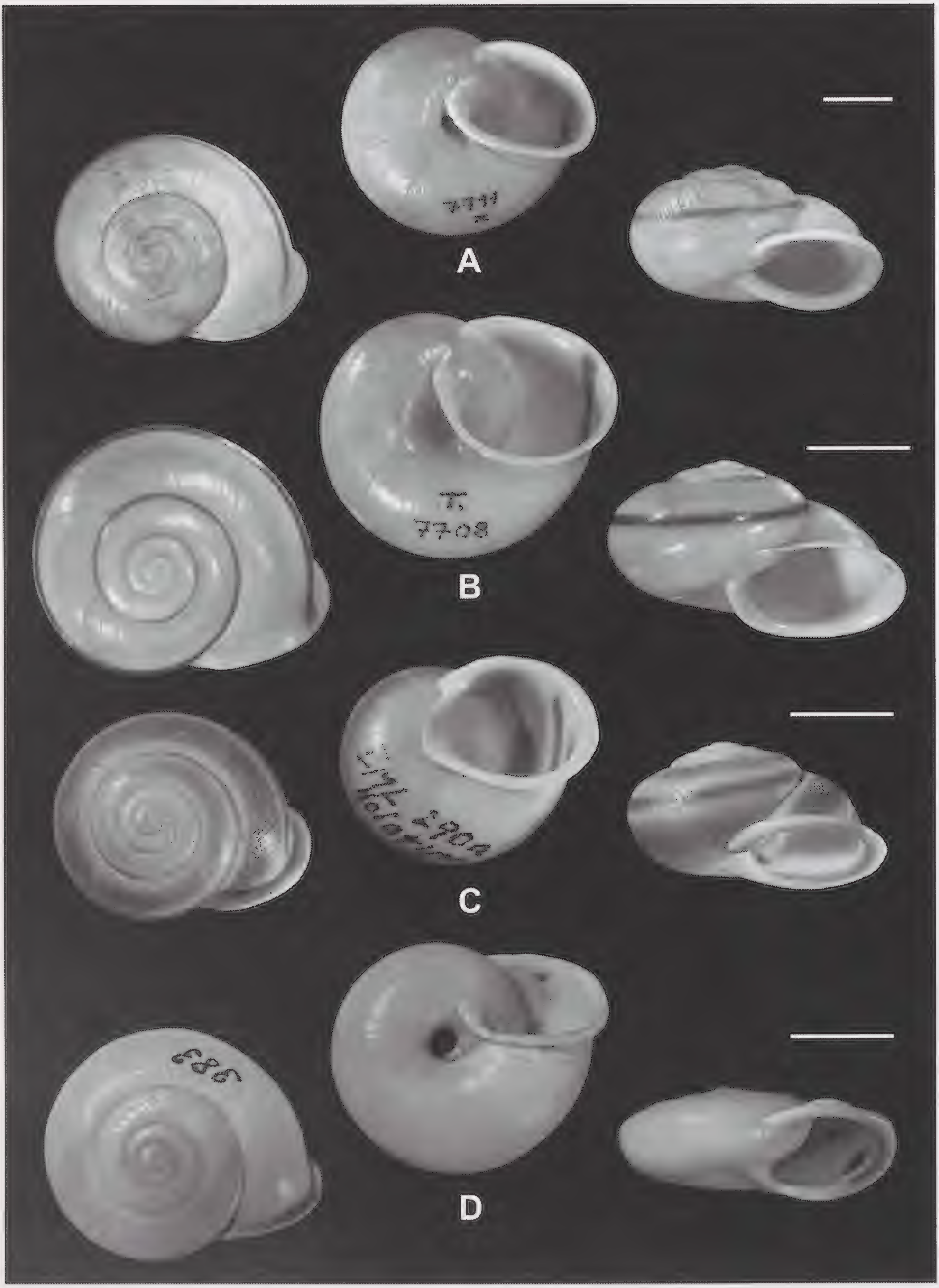


FIG. 6. Shells in dorsal, ventral and lateral view. A: *Epiphragmophora tucumanensis* (SKF 7711, lectotype). Scale bar = 10 mm; B: *Epiphragmophora trenquelleonis* (SMF 7708, lectotype). Scale bar = 10 mm; C: *Epiphragmophora variegata* (FML 290 a, holotype). Scale bar = 15 mm; D: *Epiphragmophora villavilensis* (MACN 383, holotype). Scale bar = 15 mm.

Zapallar, Hylton Scott leg. 17/II/1940. MLP 1281, Piedra Blanca, Ringuelet, R. leg., II/1949. MLP 1664, Pringles Dept., Paso del Rey, Vignatti leg. MLP unnumbered, Pringles Dept., La Toma.

Argentina, Catamarca Prov.: FML 592, El Alto, 950 m, 2/XII/1958, Hayward, K. leg. W. Weyrauch det. FML 11050, Capayan Dept., Los Angeles, 1/XI/1944, Weyrauch det.

Description:

External Features: Body dark orange, with ocular tentacle brownish towards the tip.

Shell (Fig. 6B): Dextral, helicoidal, subglobose, semisolid; spire shallow conical; medium size; 4½ convex whorls; yellowish to dark brown or chestnut; body whorl usually with one equatorial brown periphery band that also lines suture, absent on spire; shells from some localities can has a maximum of three peripheral bands, medial one more neatly delimited; protoconch smooth; teleoconch surface with very thin growth-lines or with thick regularly separated growth-ridges; fine transversal lines located between them cutting transversally axial ridges; aperture circular with white, thin, slightly reflexed peristome; parietal space between lips narrow; body whorl abruptly descending behind aperture; umbilicus deep partly overlapped by basal columelar peristomal lip; suture shallow.

Measurements: (n = 15) Dm: 25.4–34.5 mm (29.3 mm); D min: 23.1–30.0 mm (25.4 mm); H: 12.9–20.4 mm (16.3 mm); D ap: 16.1–20.9 mm (17.7 mm); H ap: 14.0–18.3 mm (15.3 mm).

Jaw: Strong, arched; four vertical ribs with regular spaces between them, margins projecting at both concave and convex jaw edges.

Genitalia (Fig. 14A–C): Terminal genitalia with dart sac complex consisting of single dart sac and two mucous glands; right gland smaller than left, round or slightly elongated, located between dart sac and vagina, distally fused with atrium wall; with thin duct located distally respect to body of gland, duct inserting below constriction of dart sac; left mucous gland oval elongated, longer than right, opening into thin duct, distally located respect to body of gland; both mucous gland ducts fused together before entering dart sac; dart sac muscular, very long, cylindrical, with distal portion thicker than proximal, both portions separated by medial constriction; dart sac naturally pleated at level of constriction; dart papillae thick, as long as distal portion of dart sac; dart sac ending at atrium level with vagina and penial complex; bursa copulatrix sac round, with very thick, short duct; sac and duct of bursa copulatrix neatly delimited; vagina long, shorter than penis; free oviduct thin, long, folded by itself, enveloped by connective tissue attaching it to bursa copulatrix; vas deferens surrounding dart sac before reaching penioviducal angle and inserting in penial complex; penial complex very long; flagellum thin, medium in length, finger-like, ending level where vas deferens enters penial complex and epiphallus begins; epiphallus shorter than penis, externally differentiated from it by abrupt change in diameter; penial retractor muscle thick, inserting in median portion of epiphallus; penis very long, cylindrical, usually four times longer than epiphallus, entering at genital atrium level with dart sac and vagina; penis usually folded by itself, with a constriction where penial sheath ends; some specimens constriction not evident, but penis notably thinner at the same point; penis sheath simple, very thick, muscular; penis sheath overlapping distal portion of penis; distal portion of penis notably thinner but together with penis sheath considerably thicker than proximal one; penial papillae short, triangular; internal distal wall of penis with thin, smooth, straight folds; proximally, close to penial papillae, penial wall with longitudinal thin pleats in zigzag pattern; vas deferens attaching to proximal penial sheath; penial muscular band absent; atrium long, with thick walls.

Habitat and Distribution: This species is widely distributed, in contrast to the majority of the *Epiphragmophora* species. *Epiphragmophora trenquelleonis* is found in Cordoba, Santiago del Estero, San Luis, Catamarca, La Rioja, Chaco and Formosa provinces, Argentina. It is also recorded for southeastern Bolivia and Paraguay. In northwestern Córdoba, *E. trenquelleonis* is more abundant than in other provinces, Argentina. Its area of distribution is included into the Chacoan biogeographic subregion.

Remarks: *Epiphragmophora trenquelleonis* is very variable in shell size and morphology. Specimens from San Luis Province, central-western Argentina, have a characteristic thinner shell and have axial ridges in the body whorl instead of the characteristic fine shell

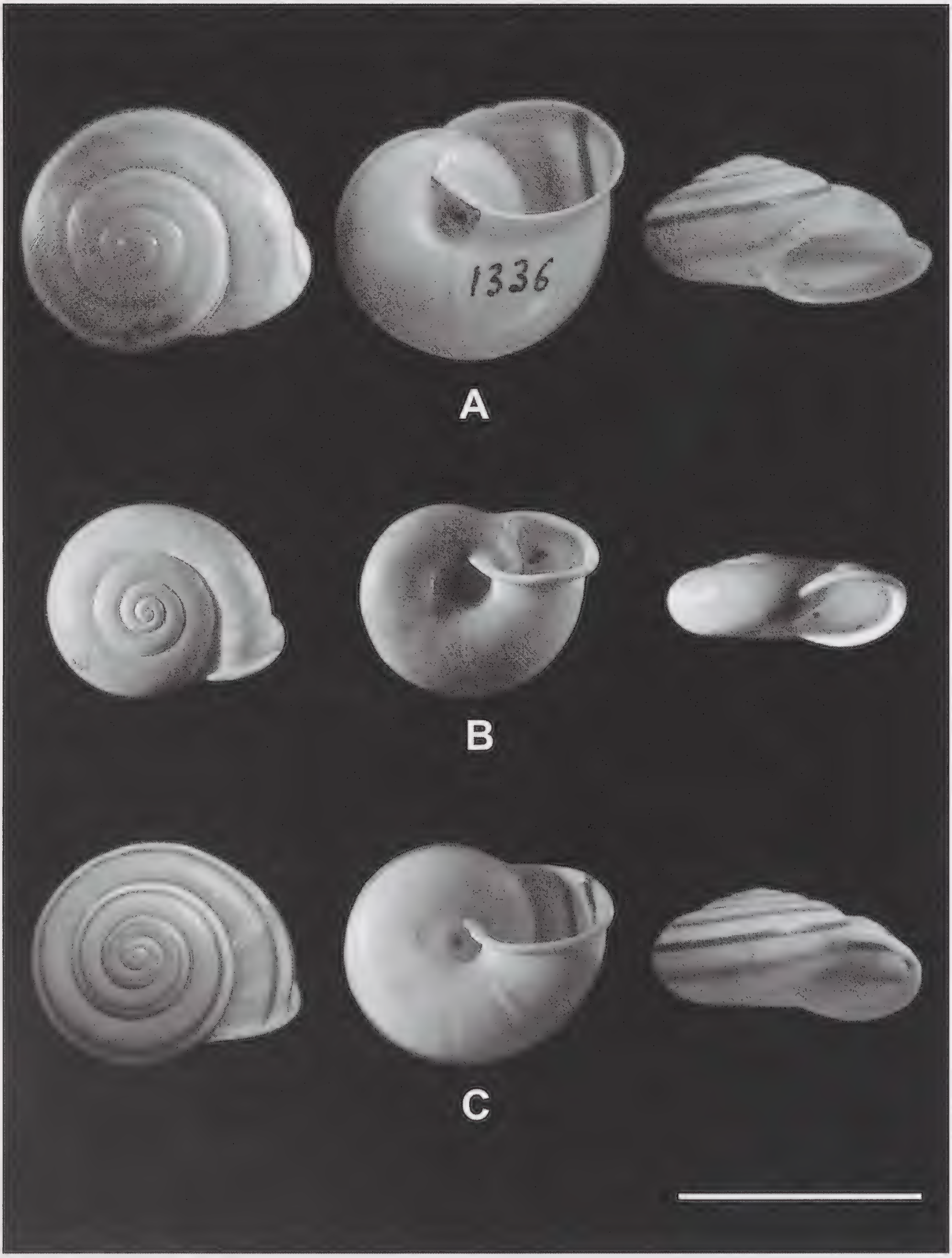


FIG. 7. Shells in dorsal, ventral and lateral view. A: ***Epiphragmophora guevarai*** (FML 14766, paratype); B: ***Epiphragmophora quirogai*** (FML 14719, paratype); C: ***Epiphragmophora walshi*** (FML 14764, paratype). Scale bar = 20 mm.

growth lines of specimens from Córdoba Province. Nevertheless, the genitalia show the same morphological pattern. Shell color is also very variable. Most noteworthy characteristics of the present species are the thick, short duct of the bursa copulatrix, a very long vagina, and the length of the penial complex, the longest of the species of *Epiphragmophora* studied. The presence of a penial constriction or a medial thinner portion is also a character not seen in other species studied. Some of the morphological variations in the genitalia are differences in vagina lengths and penial complex lengths, but the proportion between the length of the epiphallus and the penial length is constant in all specimens studied. Genitalia of *E. trenquelleonis* are similar to *E. trifasciata* Fernandez & Rumi but differ from it mainly in length of vagina, length and shape of penial complex and mucous glands. Both mentioned species, together with ***E. guevarai*** n. sp., have the shortest bursa copulatrix ducts of the genus.

Epiphragmophora trifasciata Fernández & Rumi, 1984
(Figs. 5C, 15A, B)

Epiphragmophora trifasciata – Fernández & Rumi, 1984b: 249. pl. 3, figs. 6–8, *non Helix Eurycampta trenquelleonis var. c. trifasciata* Doering, 1875a: 315.

Holotype and Paratypes: MACN 24406, Argentina, Córdoba, Agua de Oro, East Side of Sierra chica mountain, 1940, leg. José A. de Carlo.

Other Material Examined:

Dissected Material: Argentina, Córdoba Prov.: FML 14716A*, Argentina, Córdoba, Punilla Dept., Cruz Chica, 28/I/1998, Domínguez, E. leg.

Dry Material: Argentina, Córdoba Prov.: MACN 24406. FML 11011, Argentina, Córdoba, Colón Dept., between Ascochinga and El Chorrito on road from Jesús María to La Cumbre, 800 m., 25/II/1966. leg. W. Weyrauch. FML 11012, Argentina, Córdoba, El Chorrito, 1,100 m, 26/II/1966. W. Weyrauch Col. FML 942, Córdoba, Totoral Dept., Ascochinga, La Cumbre, 1,300 m, 24/XII/1967, leg. W. Weyrauch.

Description:

External Features: Body color homogeneous orange to dark red; facial groove well impressed.

Shell (Fig. 5C): Dextral, helicoidal, subglobose, solid; spire conical; medium size; 4½ convex whorls; yellowish to dark brown; body whorl with three brown peripheral bands, usually central band darker and wider than ventral and dorsal bands; protoconch smooth; surface with strong growth-ridges; suture shallow; aperture circular, with white, thin, slightly reflexed peristome; body whorl descending behind aperture; umbilicus deep, partly overlapped by peristomal lip.

Measurements: Paratypes (n = 2): D maj: 31.5 mm, 29.6 mm; D min: 26.7 mm, 28.2 mm; H: 16.8 mm, 15.4 mm; D ap: 19.5 mm, 16.6 mm.

Other Material: FML 14716 (n = 13): D maj: 28.3–32.5 mm (27.9 mm); D min: 25.0–28.3 mm (24.9 mm); H: 14.4–17.9 mm (14.7 mm).

Jaw: Arched, thin, orange; five vertical narrow ribs in central portion of the jaw.

Genitalia (Fig. 15A, B): Terminal genitalia with dart sac complex consisting of single dart sac and two unequal mucous glands; right gland small, round, located between dart sac and vagina, distally fused with atrium wall, opening into thin duct; left mucous gland cylindrical to oval with thin efferent duct running parallel to right mucous gland duct; both efferent ducts from glands distally fused with each other before entering dart sac; left mucous gland distally fused with atrial wall; dart sac long, muscular, cylindrical, with medial constriction below which mucous gland ducts inserts; dart papillae thick, cylindrical, as long as distal portion of dart sac, cylindrical papilla wrapped by connective tissue to dart sac wall ending at thin duct opening into atrium, love dart found within papilla; dart sac ending at atrium level with vagina and penial complex; bursa copulatrix sac round, with thick, very short duct; sac and duct of bursa copulatrix neatly delimited; vagina shorter than penis; penial complex short; flagellum triangular, with thick distal portion, ending level where vas deferens enters penial complex and epiphallus begins; epiphallus cylindrical, thick, shorter than penis, externally differentiated from it; penial retractor muscle inserting in median portion of epiphallus; penis cylindrical, thicker in proximal portion, gradually getting thinner

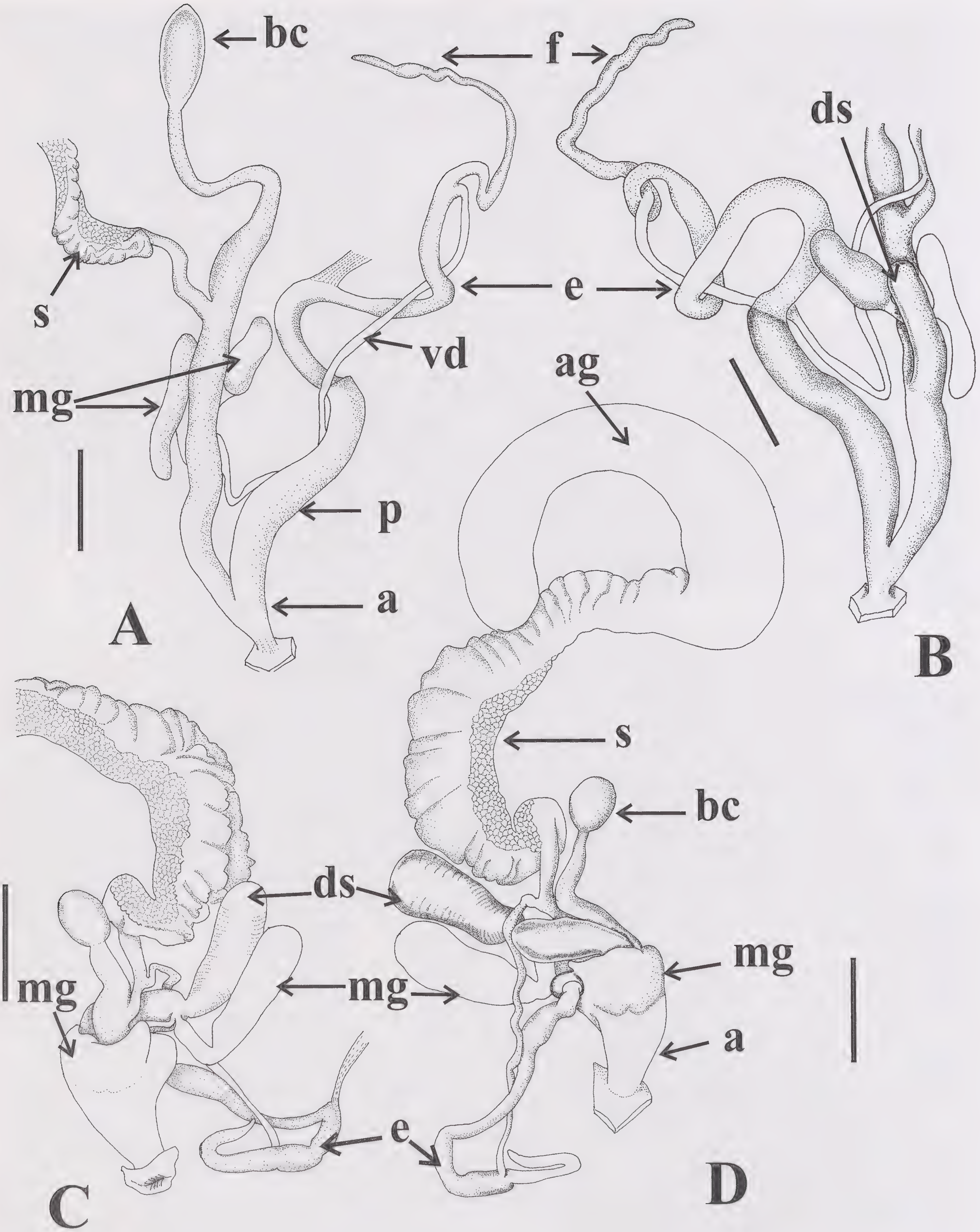


FIG. 8. *Epiphragmophora argentina and E. cryptomphala.* A: *E. argentina*, dorsal view of terminal genitalia; B: *E. argentina*, ventral view; C: *E. cryptomphala*, dorsal view of terminal genitalia, note mucous gland duct insertion; D: *E. cryptomphala*, ventral view of genitalia. Scale bars = 5 mm. Abbreviations: a, atrium; ag, albumen gland; bc, bursa copulatrix; ds, dart sac; e, epiphallus; f, flagellum; mg, mucous gland; p, penis; s, spermoviduct; vd, vas deferens.

towards distal portion, twice epiphallus length, entering at genital atrium level with dart sac and vagina; penial papillae short, located inside proximal penis; internal wall of penis with thin smooth strength pleats for all its length, some specimens with longitudinal pilaster; penis sheath simple, muscular, overlapping distal portion of penis, distally fused with atrium wall; penial muscular band absent; atrium medium in length not enveloped by mucous gland.

Habitat: *Epiphragmophora trifasciata* is found in close association to rocks or inside thin crevices between rocks.

Distribution: *Epiphragmophora trifasciata* is only known from northwestern and central Cordoba Province, Argentina, with a restricted area of distribution. Its area of distribution corresponds to the Chacoan biogeographic subregion (Morrone, 2006).

Remarks: Fernandez & Rumi (1984b) mentioned *E. trifasciata* as present in southwestern Santiago del Estero Province; nevertheless, no specimens has been found in that region nor in museum collections. For this reason, the distribution of *E. trifasciata* is now restricted to northwestern and central Córdoba Province, Argentina, based on the available data. *Epiphragmophora trifasciata* differs from *E. trenquelleonis* in shell characters such as body whorl sculpture, presence of three peripheral bands, size, and general shell shape. Differences in genitalia have been discussed in *E. trenquelleonis* and ***E. guevarai*** n. sp. descriptions.

Epiphragmophora trigrammephora
(d'Orbigny, 1835)
(Figs. 5D, 15C, D, 19, 21)

Helix trigrammephora d'Orbigny, 1835: 22.
Epiphragmophora trigrammephora – Ancey, 1897: 10.
Epiphragmophora trigrammephora – Parodiz, 1957: 30.
Epiphragmophora trigrammephora – Fernández & Rumi, 1984b: 250.

Syntypes: MNHN, Bolivia, Santa Cruz Dept., Valle Grande, 1835. BMNH, Bolivia, Santa Cruz, Pampa Grande.

Other Material Examined:

Dissected Material: Bolivia: NKC6, Santa Cruz Dept., Angel Sandoval Prov., Santo Corazón. 17°58'24"S; 58°48'28"E, NKC unnumbered, Santa Cruz Dept., Cordillera Prov., Parque Nacional Koá-Iya, 57 km O. from San José oil campament. 18°27'31"S, 61°22'53"W, 230 m, 13/VI/1998. Alfredo Fuentes leg. *FML 14784 A, Santa Cruz Dept., Valle Grande, margins of Ñancahuasu river, 19°04'41"S, 63°40'47"W, 538 m, 22/XII/2005. E. Dominguez leg. Paraguay: IBNP unnumbered, Chaco Dept., Parque Nacional Defensores del Chaco, Cerro León. 19/XI/1984. R. T. Bonace leg. IBNP unnumbered, Chaco Dept., Parque Nacional Defensores del Chaco, Cerro León, Sitio 5 hasta 8. Between 3er campament and sierra del Tigre, 16/XI/1984. J. A. Kochalka leg. Argentina, Jujuy Prov.: FML 14748 A, Santa Bárbara Dept., Sierra de Santa Bárbara, Octubre 1998. E. Dominguez leg. FML 108 A, Valle Grande Dept., on road to Valle Grande, 10/III/1989. E. Dominguez leg. FML 14749 A, Ledesma Dept., town of Yuto, margins of the river of the same name, 23°40'18"S, 64°29'44"W, 470 m, 3/IV/2004, M. G. Cuezzo leg. FML 14750 A, Valle Grande Dept., Route 83 on road to Valle Grande, 23°31'46"S, 64°57'47"W, 1,290 m, 3/IV/2004, M. G. Cuezzo leg. FML 14751 A, Ledesma Dept., Calilegua National Park, 23°45'36"S, 64°51'02"W, 700 m, 2/IV/2004, C. Nieto leg. MLP unnumbered, Jujuy Prov., Santa Bárbara Dept., El Palmar, 15/II/1947, Birabén leg. Argentina, Salta Prov.: FML 14752 A, Route 9, on road to El Carmen from Salta to Jujuy, 24°26'43"S, 65°17'59"W, 1,230 m, 18/XII/2001, M. G. Cuezzo leg. & det. MLP unnumbered, Gral. José de San Martín Dept., 27/XI/1954.

Dry Material: Argentina, Jujuy Prov.: FML 14737, Ledesma Dept., 6 km from Valle Grande, 23°30'39"S, 64°57'12"W, 1,570 m, 3/IV/2004, M. G. Cuezzo leg. & det. FML 14738, Ledesma Dept. town of Yuto, margins of Yuto river, 23°40'18"S, 64°29'44"W, 500 m, 3/IV/2004, M. G. Cuezzo leg. & det. FML 544, km 54 on road from Jujuy to San Pedro, 720 m, 24/II/1962. leg. & det. W. Weyrauch. FML 1385, San Pedro Dept., San Pedro, leg. P. Wygodzinsky, det. W. Weyrauch. FML 10, between Chalican and Quemado, 27/VII/1948. leg. A. Castellanos, det. Hylton Scott. FML 11055, Las Capillas, 36 km NW from Jujuy, 1,200 m, 25/II/1964.

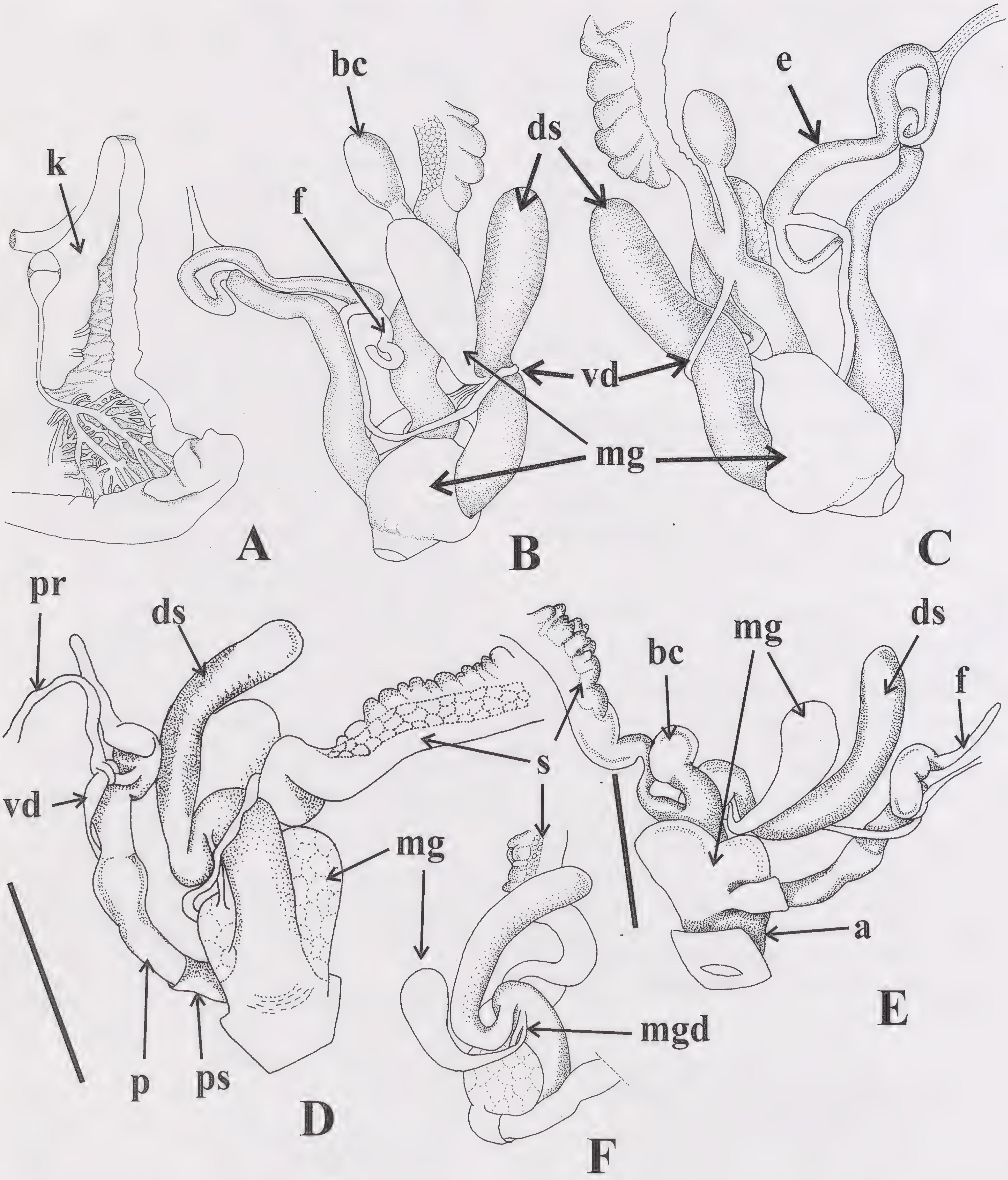


FIG. 9. *Epiphragmophora escoipensis* and *E. hieronymi*. A: *E. escoipensis*, pallial system; B: *E. escoipensis*, ventral view of terminal genitalia, note the point of insertion of both mucous glands ducts; C: *E. escoipensis*, dorsal view of terminal genitalia; D: *E. hieronymi*, ventral view of terminal genitalia; E: *E. hieronymi*, dorsal view of terminal genitalia, mucous gland is partially folded to see the fusion of both mucous glands ducts into one inserting in dart sac; F: *E. hieronymi*, lateral view of terminal genitalia shows point of insertion of mucous glands. Scale bars = 5 mm. Abbreviations: a, atrium; bc, bursa copulatrix; ds, dart sac; e, epiphallus; f, flagellum; k, kidney; mg, mucous gland; mgd, mucous gland duct; p, penis; ps, penis sheath; pr, penial retractor; s, spermoviduct; vd, vas deferens.

leg. & det. by W. Weyrauch. FML 926, La Cienaga, San Pedro, 3/III/1955, J. Foester leg. MLP 4258, El Aibal, Prosen, A. leg. I/1948. MLP 4266, Las Capillas, Biraben, M. 26/XI/1950. MLP unnumbered, San Pedro Dept., Las Quinas, La Mendieta, Birabén, M. 2/VI/1947. MLP unnumbered, El Palmar, Santa Bárbara, V/1947, ex Hylton Scott collection. MLP unnumbered, San Pedro Dept., La Mendieta, Birabén, M., V/1947, ex Hylton Scott collection. FML 901, 20 km South of Urundel, 400 m, 26/II/1964 leg. & det. by W. Weyrauch. FML 14736, Ledesma Dept. between Calilegua and Valle Grande, E. Dominguez leg. Argentina, Salta Prov.: FML 14365, Capital Dept., 13 km from Salta on road to Güemes, 1,300 m, 20/X/1968. leg. & det. by W. Weyrauch. FML 849, Orán Dept., 8 km N of Urundel, 350 m, 26/II/1964. leg. & det. W. Weyrauch. FML 827, Cerro Colorado, Puesto Pala-pala, 650 m, 21/II/1964, W. Weyrauch leg. & det. FML 633, Orán Dept., Urundel, 27/II/1949, Huek leg., W. Weyrauch det. FML 14735, Los Colorados, X/1993, F. Cruz & G. Scrocchi leg. FML 981, Gral. José de San Martín Dept., 10 km S of Embarcación, 300 m, leg. & det. by W. Weyrauch. FML 389, Güemes Dept., Cerro Pelado, 1,500 m, 4/I/1956. E. de la Sota leg. MLP unnumbered, Gral. Jose de San Martín Dept., Vespucio, ex Hylton Scott col. MLP unnumbered, Orán Dept., Urundel, ex Hylton Scott collection. MLP unnumbered, Carapacú, Bridarolli leg. 1945, ex Hylton Scott collection. MLP unnumbered, Anta Dept., Luis Burela, Birabén, M. leg. 20/XI/1954, ex Hylton Scott collection. MLP unnumbered, Termas del Palmar, Ramos de Martines leg. 10/I/1950, ex Hylton Scott collection. MLP unnumbered, Santa Victoria Dept., Cerro Colorado, Apostol, I. leg., I/1962. MLP 2152, Quebrada del Carapato, Birabén, M. leg., 22/XI/1951. MLP 1644, Gral. José de San Martín Dept., General Ballivian. MLP 4267, San Pedro, Birabén, M. 29/XI/1957. Argentina, Santiago del Estero Prov.: MACN 23037, Cerro del Remate, 1936, R. Schreiter leg. Argentina, Formosa Prov.: MLP 4264, Laguna Yema, Birabén, M. leg. 19/V/1947. MLP 4261, Ing. G. Juarez, M. Birabén, leg., XII/1954. Bolivia: FML 2241, 4–5 km W of Villa Montes, 400–500 m 6/VII/1969. leg. & det. by W. Weyrauch. FML 768, mountains W of Santa Cruz. leg. & det. by W. Weyrauch. MLP unnumbered, Santa Cruz, Rio Seco. 25/I/1964, Birabén, M. ex Hylton Scott collection. FML 240, Santa Cruz, 16/II/1947, Pierotti leg., W. Weyrauch det.

Description:

External Features: Uniform pale yellow to light gray; dorsal row of pustules between ommatophores well delimited.

Shell (Fig. 5D): Dextral, helicoidal, subglobose, semisolid; usually with high conical spire; medium size; 4¾ to 5¾ convex whorls; pale cream to yellow; three peripheral thin, darker brown bands clearly delimited on body whorl, upper band running centrally in each anfractum of spire, usually central band slightly wider, more darkely pigmented than others, always with well defined limits; protoconch smooth, first whorls with thin growth lines; teleoconch surface generally malleated, with diagonal ribs and transverse growth lines interrupted by axial ribs on body whorl, in some specimens body whorl with thick axial growth lines instead of malleated surface; suture deep impressed; aperture subquadrangular or subovoidal; peristome white, thin, delicate, expanded, slightly reflexed; body whorl abruptly descending towards aperture; umbilicus deep, wide.

Measurements: Syntypes (n = 2): D maj: 23.6 mm, 24.9 mm (24.2 mm); D min: 20.6 mm, 21.2 mm (20.9 mm); H: 12.6 mm, 13.5 mm (13.0 mm); D ap: 13.4 mm, 13.6 mm (13.5 mm); H ap: 11.9 mm, 12.3 mm (12.1 mm).

Jaw: Arched, thin, orange; four vertical narrow ribs in central portion of jaw with margins projecting at convex jaw edge.

Pallial Complex: Lung roof without dark spots, homogeneously pale cream; externally two to three shell peripheral bands conspicuously marked on distal portion of lung roof and mantle collar; kidney extending 50% of total pulmonary roof length; main pulmonary vein splitting proximally into two mayor veins that continue without further divisions until reaching mantle collar; portion of lung roof between rectum and kidney crossed by several minor transversal ramified veins, shallowly marked.

Genitalia (Fig. 15C, D): Terminal genitalia tight packed by connective tissue; presence of single dart sac and two mucous unequal glands, both sac-like, generally elongated, voluminous, enveloping distal vagina and dart sac, distally fused with atrium wall; each mucous gland opening into individual, thin, efferent duct that fuse together before inserting at same point below constriction of dart sac;

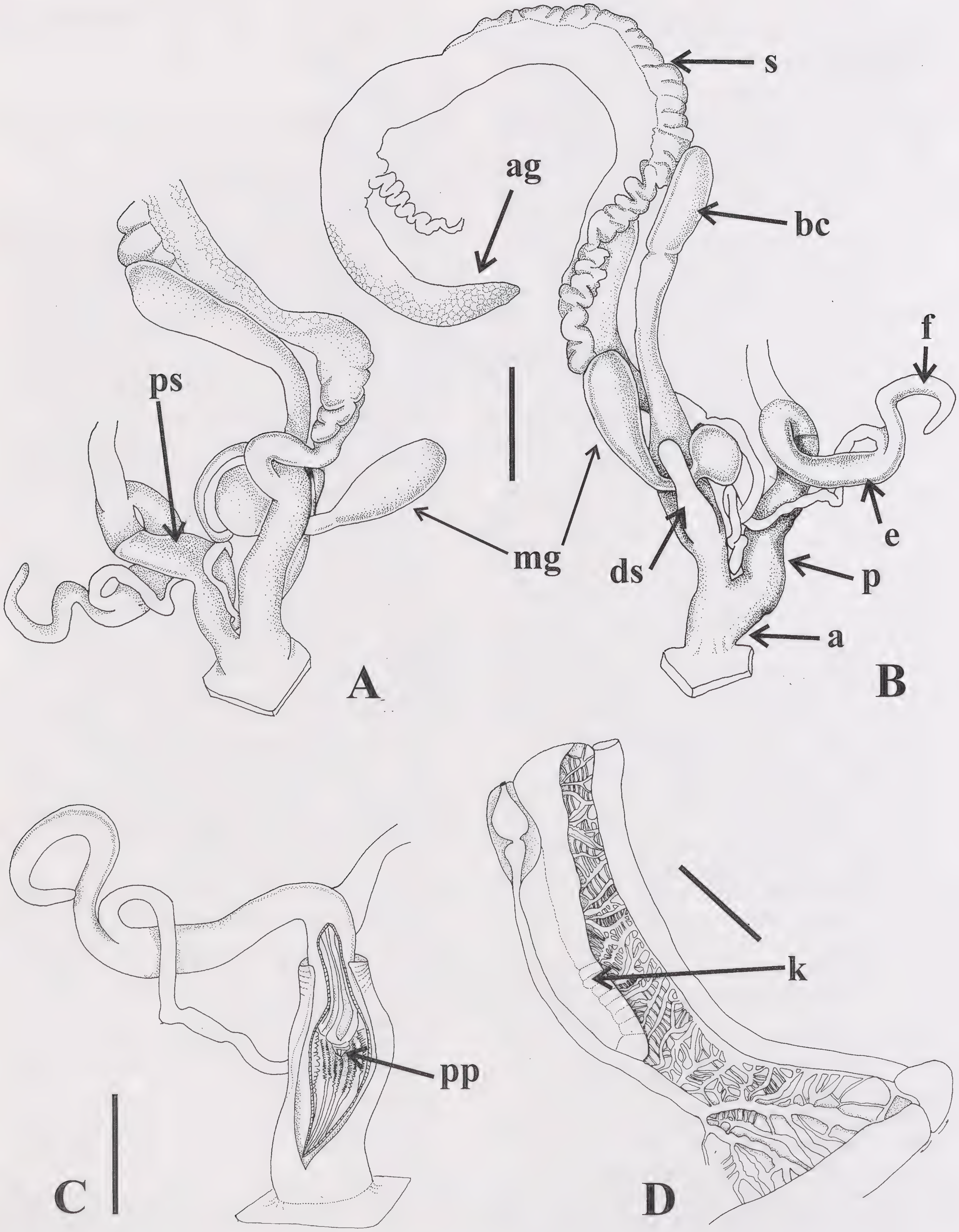


FIG. 10. *Epiphragmophora hemiclausa.* A: Ventral view of terminal genitalia; B: Dorsal view of genital system; C: Detail of penial complex; D: Pallial system. Scale bars = 5 mm. Abbreviations: a, atrium; ag, albumen gland; bc, bursa copulatrix; ds, dart sac; e, epiphallus; f, flagellum; k, kidney; mg, mucous gland; p, penis; pp, penial papilla; ps, penis sheath; s, spermoviduct.

dart sac muscular, with thick walls, long, cylindrical, with constriction located in basal $^{1}/_{3}$ of total length, distal portion of dart sac longer than proximal; dart sac naturally pleated by itself at level of constriction; vas deferens surrounding dart sac level with constriction before inserting in penial complex; dart papillae half length of dart sac distal portion; dart sac ending at atrium level with vagina and penial complex; bursa copulatrix sac round, with extremely short, thick duct; sac and duct of bursa copulatrix neatly delimited; vagina shorter than penis; free oviduct short, enveloped by connective tissue together with bursa copulatrix; penial complex medium to short in total length; flagellum thin, finger-like, ending level where vas deferens enters penial complex and epiphallus begins; proximal portion of epiphallus widening at point of entrance of vas deferens; epiphallus shorter than penis, externally differentiated from it; penial retractor muscle inserting in median portion of epiphallus; penis cylindrical, double length of epiphallus, entering at genital atrium level with dart sac and vagina; penial papillae short, triangular in proximal penis; internal wall of penis with thin, smooth, straight folds; penis sheath muscular overlapping distal portion of penis, distally fused with atrium wall; atrium long.

Habitat: Under or below tree logs or under rocks, from 200 to 1,600 m above sea level.

Distribution (Figs. 19, 21): In Argentina, *E. trigrammephora* inhabits the Yungas and Chaco biogeographic provinces. Its distribution ranges the provinces of Jujuy, Salta, Formosa, Chaco, and Santiago del Estero. In Bolivia and Paraguay, it lives in dryer habitats in the Chacoan biogeographic subregion and within transition habitats from dry forest to more humid rainforest of Yungas.

Remarks: *Epiphragmophora trigrammephora* shows high variability in spire height; although always conic, it is shallow in populations of some localities of Salta and Santiago del Estero provinces, Argentina. However, the spire is generally taller and conic in specimens from Bolivia and other localities in northern Argentina. The umbilicus is always perspective, deep, but the diameter is variable. The three peripheral bands are not always present, with some specimens having two or even only one band. In general, most of the specimens have thicker shell peripheral bands than in ***E. walshi*** sp. nov.

Epiphragmophora tucumanensis (Doering, 1874)
(Figs. 6A, 14D, E, 19)

Helix Group *Aglaja estella tucumanensis* Doering, 1874: 445.
Helix tucumanensis – Kobelt, 1878: 148.
Helix tucumanensis – Tryon, 1888: 77.
Epiphragmophora tucumanensis – Pilsbry, 1894: 198.
Helicigona catamarcana Ihering, 1910: 423.
Epiphragmophora tucumanensis – Parodiz, 1957: 30.
Epiphragmophora tucumanensis – Fernández & Rumi, 1984b: 251.

Lectotype: SKF 7711, Argentina, Tucumán [en la pendiente del Oeste de la Sierra de Tucumán, en bosques exuberantes, a 500–900 mts. de altura]. Stelzner leg. Doering det.

Other Material Examined:
Dissected Material: Argentina, Tucumán Prov.: FML 14413, Tafí del Valle Dept., route 307 between El Mastil and Tafi del Valle, 26°58'06"S, 65°39'24"W, 1,741 m, 21/XII/2001, M. G. Cuezzo leg. FML 123, Chicligasta Dept., Cochuna, 1,130 m, M. G. Cuezzo leg. FML 124, Tafi del Valle Dept., route 307 before reaching El Mastil, 6/V/1995, E. Dominguez leg. *FML 102, Tafi del Valle Dept. “El Indio”, 963 m, 15/IV/1989, M. G. Cuezzo leg.
Dry Material: Argentina, Tucumán Prov.: *FML 755, Tafí del Valle Dept., Valley of river Los Sosa, 650–900 m, W. Weyrauch leg. & det. *FML 762, Tafi del Valle Dept., Los Sosa river, 700–900 m, 16/XI/1963, W. Weyrauch leg. FML 527, Burruyacú Dept., Alto de Medina, 1,400 m, 18/II/1962, W. Weyrauch leg. & det. FML 200, Quebrada de La Hoyada, det. W. Weyrauch. FML 164, Quebrada del Saladillo, 15/X/1933, R. Schreiter leg. FML 756, Burruyacú Dept., Alto de Medina, 1,400 m, 7/II/1967, W. Weyrauch leg. & det. FML 758, Tafí Viejo Dept. San Javier Mountains, 1,000 m 12/VIII/1967, W. Weyrauch leg. & det. FML 1383, Aconquija, 6/VI/1948, Wygodzinsky leg., W. Weyrauch det. FML 1393, Tafi Viejo Dept., Horco Molle near Tucumán city, 700 m,

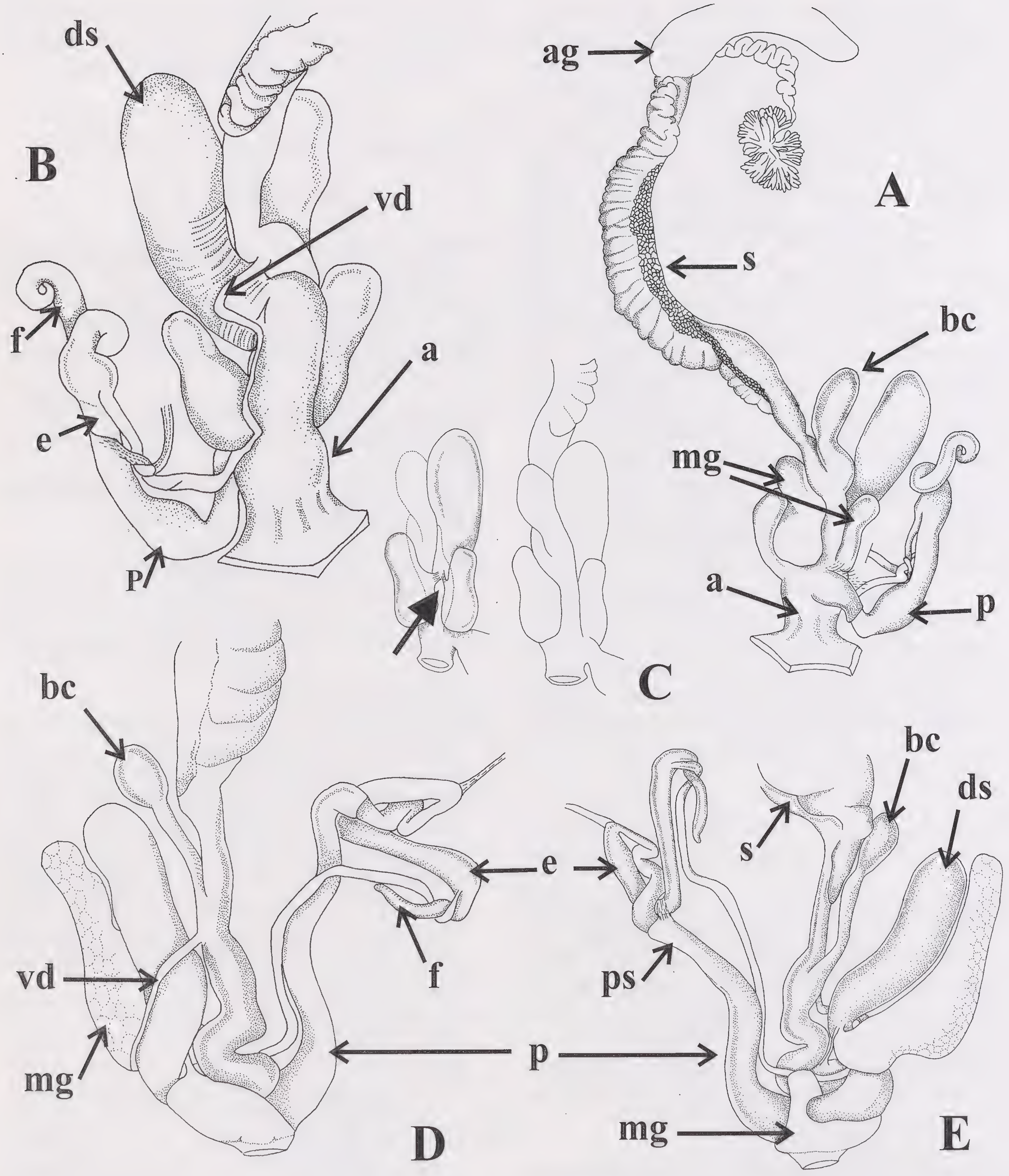


FIG. 11. *Epiphragmophora jujuyensis* and *E. parodizi*. A: *E. jujuyensis*, dorsal view of genitalia; B: *E. jujuyensis*, detail of dart sac complex in dorsal view of terminal genitalia; C: *E. jujuyensis*, detail of mucous gland ducts insertion with and without drawn of bursa copulatrix, arrow indicates point of insertion; D: *E. parodizi*, dorsal view of terminal genitalia; E: *E. parodizi*, ventral view of terminal genitalia. Scale bars = 5 mm. Abbreviations: a, atrium; ag, albumen gland; bc, bursa copulatrix; ds, dart sac; e, epiphallus; f, flagellum; mg, mucous gland; p, penis; ps, penis sheath; s, spermoviduct; vd, vas deferens.

4/II/1968, W. Weyrauch leg. & det. FML 11310, Tafi Viejo Dept., Taficillo, 18/VIII/1966, W. Weyrauch det. MACN 9009, Aconquija. MACN 608, Aconquija. MLP 4273, Tafi Viejo Dept. Horco Molle. MLP 4274, Cuesta del Clavillo, Aconquija.

Description:

External Features: Animal light to dark brown; homogeneous color over cephalopedal region; two lines of pustules separated by groove running dorsally from mantle collar to cephalic region, ending between ommatophores; dorsal groove in pedal region conspicuously marked.

Shell (Fig. 6A): Dextral, helicoidal, globose, solid; elevated conical spire; medium to large size; 4¾ to 5 convex whorls; color variable from yellowish to dark brown; presence of single thick supraequatorial, dark brown, even, band most notable in body whorl periphery; protoconch smooth; teleoconch with axial ridges, surface of body whorl strongly malleated to reticulated; suture deep impressed; aperture subcircular to ovoid; peristome whitish, thick, reflexed; some specimens with basal lip thickened; body whorl distinctly descending towards aperture; umbilicus narrow, partly overlapped by margins of peristomal lips.

Measurements: Specimens from Argentina, Tucumán, Burruyacú Dept., Alto de Medina (n = 10): DM: 33.7–29.6 mm (31.9 mm); D min: 28.9–25.0 mm (27.3 mm); H: 18.6–15.5 mm (17.2 mm); D ap: 19.9–17.3 mm (19.0 mm); H ap: 16.0–14.3 mm (15.2 mm).

Specimens from Argentina, Tucumán, Yerba Buena Dept., San Javier, (n = 8): DM: 30.0–28.0 mm (28.4 mm); D min: 25.8–23.2 mm (24.5 mm); H: 16.5–14.2 mm (15.8 mm); D ap: 18.5–16.2 mm (17.3 mm); H ap: 15.0–13.5 mm (14.3 mm).

Specimens from Argentina, Tucumán, Tafi del Valle Dept., margins of Los Sosa river (n = 6): DM: 41.9–32.8 mm (37.3 mm); D min: 36.4–27.6 mm (32.1 mm); H: 29.3–17.7 mm (23.9 mm); D ap: 24.9–19.4 mm (22.1 mm); H ap: 19.8–15.6 mm (17.8 mm).

Pallial Complex: Lung roof heavily mottled with black spots lining most of the veins; several minor veins located between rectum and kidney; kidney extending half of pulmonary roof length; main pulmonary vein splitting into two major veins in distal lung roof below kidney and several minor longitudinal veins.

Jaw: Arched, generally narrow, orange, with four thin central ribs, margins projecting at both edges.

Genitalia (Fig. 14D, E): Terminal genitalia with dart sac complex consisting of single dart sac and two unequal mucous glands symmetrically located on either side of dart sac; left mucous gland oval-elongated; right gland oval to cylindrical; both glands with thin efferent ducts inserting independently in middle zone of dart sac; duct of left mucous gland departing from middle portion of body gland; duct of right mucous gland distal respect to body of gland; dart sac muscular, short, cylindrical, with basal portion slightly wider in diameter, or short cylindrical and even in diameter, inserting in middle portion of vagina; dart sac without medial constriction; internal wall of dart sac with uniform longitudinal bands, dart papillae short, located in distal dart sac; bursa copulatrix elongate-oval; duct of bursa copulatrix medium length, running parallel to distal portion of spermoviduct; penial complex long; flagellum long, thin, convoluted; transition between epiphallus and penis marked by abrupt increase in diameter of penis; internal wall sculpture of epiphallus consisting on straight longitudinal bands; penis half length of epiphallus; internal wall sculpture of proximal penis with thin zigzag longitudinal bands, distal penis with closed columns in zigzag pattern ending in straight bands; penial papillae absent; penial sheath double, internal one muscular fused with atrium, running to middle penis; external penis sheath transparent, thin, enveloping penis in its complete length; penial muscular band long, located under penial muscular sheath; penial retractor muscle thick, inserting in middle portion of epiphallus; penis and vagina entering side by side at atrium, distally to dart sac complex; atrium long.

Habitat: *Epiphragmophora tucumanensis* inhabits subtropical rainforests of Yungas Province from 500 m to 1,800 m above sea level, where the snails are found under tree logs or actively crawling on dead leaves on the ground.

Distribution (Fig. 19): *Epiphragmophora tucumanensis* is distributed mainly in Tucumán Province, northwestern Argentina. Some scattered records exist in Catamarca Province.

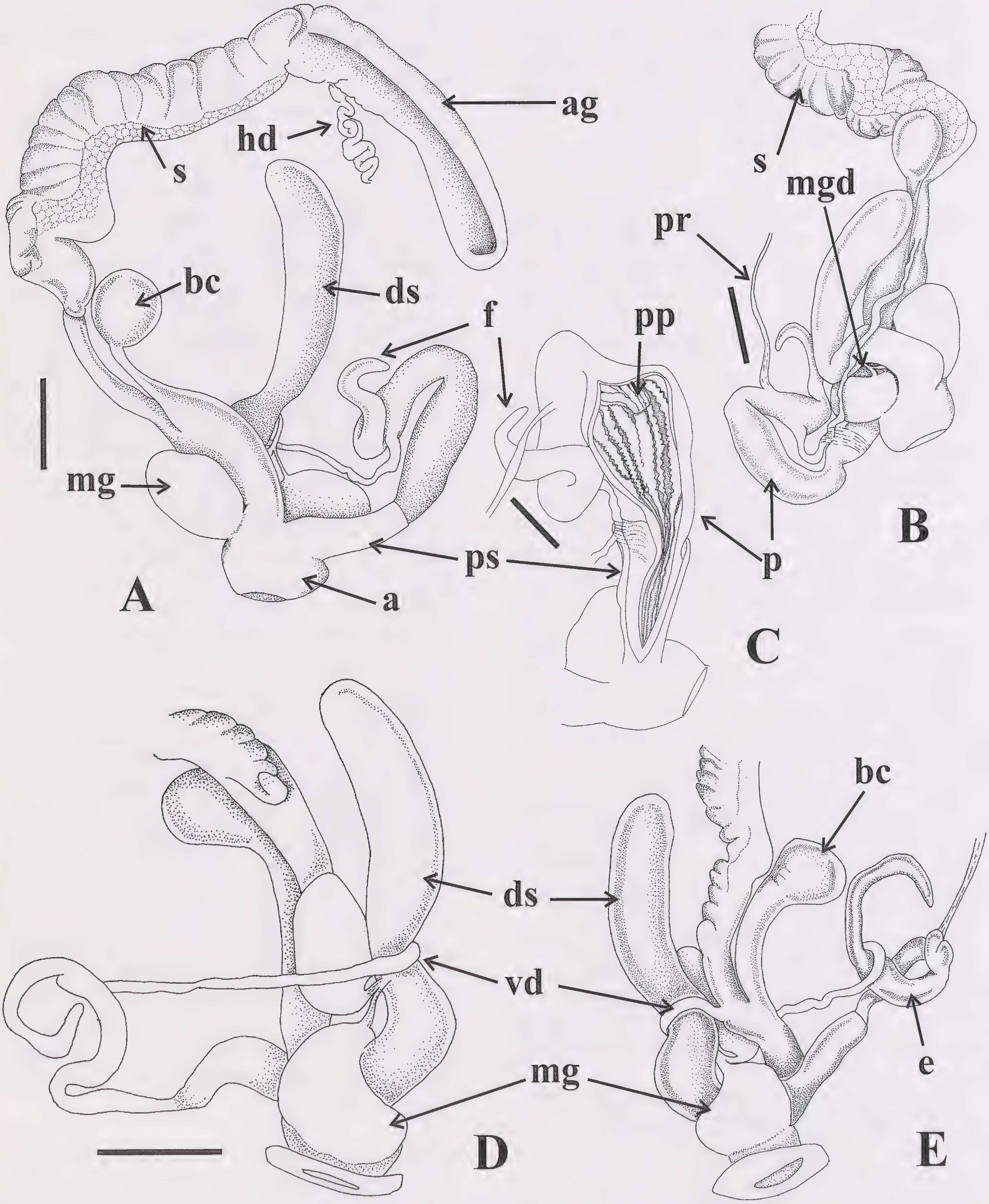


FIG. 12. *Epiphragmophora puntana* and *E. rhathymos.* A: *E. puntana*, ventral view of genitalia; B: *E. puntana*, detail of dorsal view of terminal genitalia; C: *E. puntana*, detail of penial complex showing internal sculpture of penis; D: *E. rhathymos*, ventral view of terminal genitalia; E: *E. rhathymos*, dorsal view of terminal genitalia. Scale bars = 5 mm. Abbreviations: a, atrium; ag, albumen gland; bc, bursa copulatrix; ds, dart sac; e, epiphallus; f, flagellum; hd, hermaphroditic duct; mg, mucous gland; mgd, mucous gland ducts; p, penis; pp, penial papillae; ps, penis sheath; s, spermoviduct; vd, vas deferens.

Remarks: *Epiphragmophora tucumanensis* (Doering) was originally described as a variety of *Helix estella* d'Orbigny, 1835. *Epiphragmophora estella* differs from *E. tucumanensis* in its smaller shell size, shell sculpture, less convex whorls and color. Unfortunately, the anatomy of *E. estella* is still unknown.

The type locality of the present species is confusing because the locality "Sierra de Tucumán", is imprecise, no other detail of the type locality has been recorded. Indeed, Tucumán Province has pre-Andean mountains often called as "sierras", running about 200 km in its western part from north to south. Because of this vague information on the type locality, some authors had considered San Javier, in the central to northern portion of the western mountains in Tucumán, as the type locality of *E. tucumanensis*, whereas others mentioned both San Javier and Tafi del Valle as the type locality. Considering *E. tucumanensis* distribution and the abundance of specimens between 500–900 m, I interpreted that Doering's type locality would be the mountains on the road to Tafi del Valle, from 500 to 900 m, on the western side of the mountains where the species is found in humid cloud forest of the Yungas biogeographic region. Specimens of Tafi del Valle are usually darker with shell size remarkably bigger than specimens of other localities. This is the only species of the genus for which the morphology of the spermatozoon has been investigated (Cuezzo, 1994).

Epiphragmophora variegata Hylton Scott, 1962
(Figs. 6C, 16A–D, 20)

Epiphragmophora variegata Hylton Scott, 1962: 105

Epiphragmophora variegata – Fernández & Rumi, 1984b: 252.

Holotype: FML (ex IML) 290a, Argentina, Catamarca, Dept. Belén, Agua de Dionisio, Farallón Negro, 1953.

Other Material Examined:

Dissected Material: Argentina, Tucumán Prov.: FML 14710 A, J. B. Alberdi Dept., Dique Escaba, Escaba Abajo, 27°38'17"S, 65°47'43"W, 1,640 m, 10/XI/1999, Dominguez leg. FML 14713 A, J. B. Alberdi Dept., Dique Escaba, 700 m, 27°40'03"S, 65°45'00"W, 24/IV/2003, M. G. Cuezzo leg. & det. FML 14712 A, J. B. Alberdi Dept., on road to Dique Escaba, 5 km from Alberdi, 1/XI/1996, M. G. Cuezzo leg. & det. Argentina, Catamarca Prov.: FML 14714 A, Andalgalá Dept., Las Estancias, El Lindero, 1 km from the Hostal Aconquija, 27°28'07"S, 66°00'37"W, 1,530 m, 19/IV/2003, M. G. Cuezzo leg. & det.

Dry Material: Argentina, Tucumán Prov.: FML 14366, Dique San Ignacio, 600 m, 17/V/1964, leg. & det. by W. Weyrauch. FML 14367, FML 709, San Ignacio river, 420 m, 4/XII/1966, leg. W. Weyrauch. FML 702, Dique Escaba, 5/XII/1966, leg. W. Weyrauch. FML 153, Santa Ana Mountain, 1/VII/1946, leg. R. Schreiter, det. W. Weyrauch. FML 973, Huasa-Pampa, 500 m, 9/V/1964, Arevalo leg. FML 400, Dique Escaba, 4/VI/1956, leg. Tomsic, det. W. Weyrauch. FML 289, Rio Chico Dept., close to river Marapa, 8/VI/1953, S. A. Pierotti leg.

Argentina, Catamarca Prov.: FML 290*, Hualfin Dept., Aguas de Dionisio, Farallón Negro, 16/X/1953, S. A. Pierotti leg., J. J. Parodiz det. FML 862, Las Viñas, 23/II/1964, Frias and Rodriguez leg. FML 5219, FML 10939, Las Estancias, 1,500 m, leg. & det. by W. Weyrauch

Description:

External Features: Specimens with several different body colors, from pale cream, yellow, orange, reddish to dark brown; there is no relationship between body and shell color, both of which are highly variable; dorsal row of pustules between ommatophores well delimited.

Shell (Fig. 6C): Dextral, helicoidal, globose, solid; medium to small; with high conical spire; 4½ convex whorls delimited by a well-impressed suture; shell color highly variable, from uniform pale yellow to dark brown; generally with three pigmented peripheral bands, dorsal and ventral bands wider than equatorial, about 2 mm in thickness, dark brown, neatly delimited, bands evident in body whorl persisting only dorsal band in spire; dorsal and ventral bands in some specimens with different color than central band, usually lighter; few specimens with one equatorial band; protoconch smooth; teleoconch with fine growth lines; surface of body whorl malleated; aperture subcircular with a thick, white, strongly reflexed peristome; callus between margins of dorsal and ventral lip usually raised; body whorl abruptly descend-

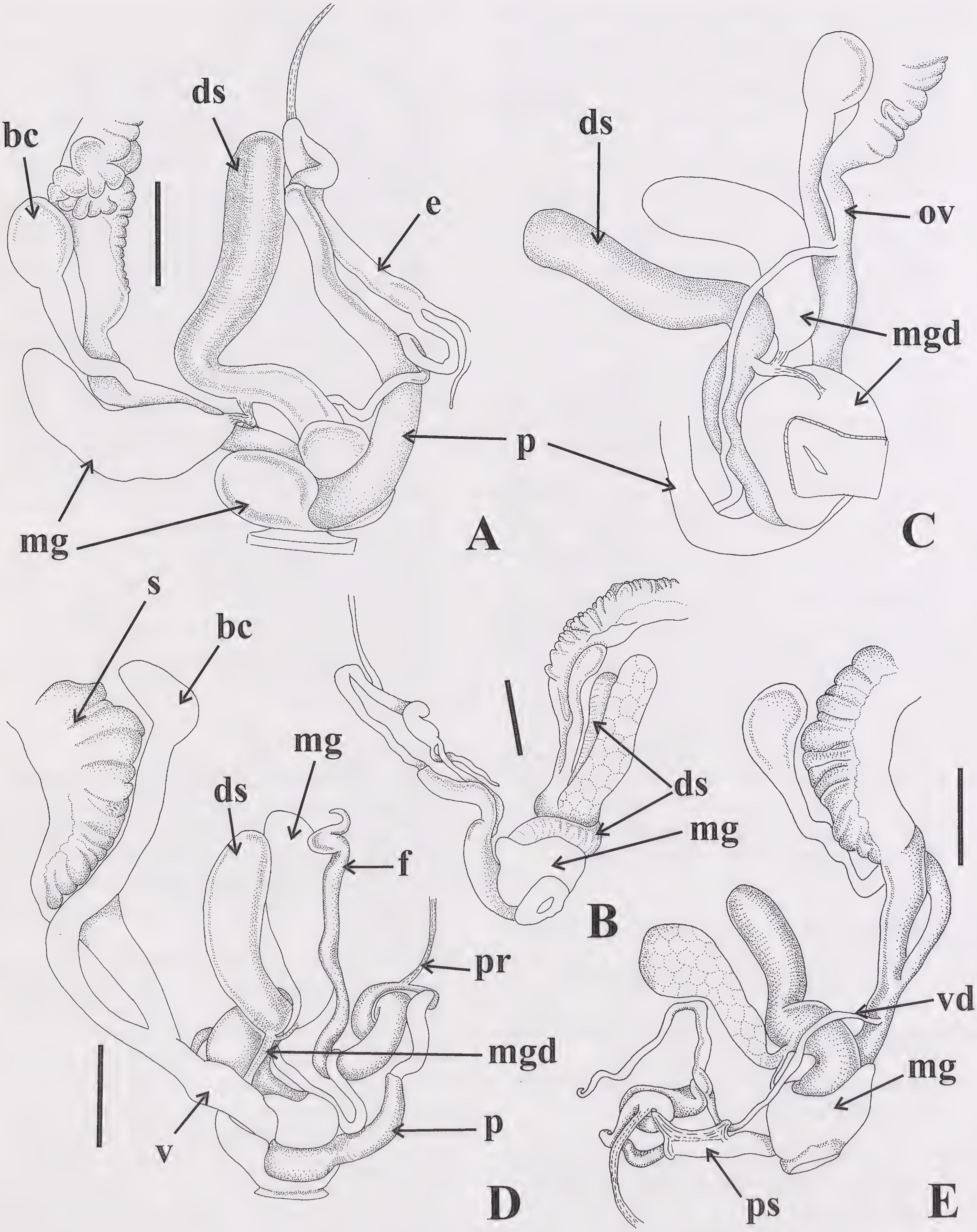


FIG. 13. *Epiphragmophora saltana* and *E. tomsici*. A: *E. saltana*, dorsal view of terminal genitalia; B: *E. saltana*, ventral view of terminal genitalia showing union of both mucous glands ducts; C: *E. saltana*, general view of terminal genitalia inverse side; D: *E. tomsici*, dorsal view of terminal genitalia; E: *E. tomsici*, idem, ventral view. Scale bars = 5 mm. Abbreviations: bc, bursa copulatrix; ds, dart sac; e, epiphallus; f, flagellum; mg, mucous gland; mgd, mucous gland ducts; ov, free oviduct; p, penis; ps, penis sheath; s, spermoviduct; v, vagina; vd vas deferens.

ing behind aperture; umbilicus completely overlapped by columelar portion of peristome but not fused with shell wall in most specimens, only in some specimens basal lip partially sealed to shell wall with umbilicus still visible.

Measurements: Holotype: D maj: 25.5 mm; D min: 22.1 mm; H: 15.7 mm.
Specimens from Escaba, Tucumán (n = 20): D maj: 21.8–32.2 mm (26.3 mm); D min: 20.0–27.3 mm (23.1 mm); H: 13.5–23.3 mm (17.6 mm).

Jaw: Arched, orange; number and width of vertical ribs in central portion of jaw highly variable, between two to five.

Pallial Complex: Lung roof dark, heavily mottled with small black spots between major and minor veins, dark pattern of color attenuated in some specimens; main pulmonary vein splitting into two main branches before reaching mantle collar; several minor branches departing from main veins; portion of lung roof between rectum and kidney crossed by several transverse minor veins; kidney as long as half pulmonary roof length.

Genitalia (Fig. 16A–D): Terminal genitalia with dart sac complex consisting of single dart sac and two mucous glands unequal in size; right mucous gland oval, thinn; left mucous gland pea-shaped, both gland efferent ducts ending independently at middle zone of dart sac; dart sac cylindrical, muscular, smaller than mucous glands; dart papilla small, located in basal portion of dart sac; dart sac complex ending at distal portion of vagina; penial complex and vagina ending level at atrium and distal with respect to dart sac; bursa copulatrix as long as half spermoviduct length; sac of bursa copulatrix oval, sharply differentiated from duct; vagina long, slightly less longer than penis, with internal longitudinal bands; penial complex long; flagellum short to medium, finger-like, $^1/_4$ length of epiphallus + penis; epiphallus slightly thinner than penis; penis sheath muscular, overlapping all penis length, distally fused to atrium wall; penial papilla absent; penial internal wall sculpture consisting of zigzag longitudinal pleats; atrium short to medium, internal wall with longitudinal bands wider than those in vaginal internal wall.

Habitat: On rocks walls in mountains or among grass in the southern portions of the cloud rainforest region, Yungas biogeographic province (Fig. 24).

Distribution (Fig. 20): *Epiphragmophora variegata* occurs from southern Tucumán to southern Catamarca provinces, northwestern Argentina. Specimens are very abundant in La Cocha and J. B. Alberdi departments, Tucumán Province.

Remarks: The present species is highly variable in shell color with eight different patterns of banding and color at the same locality, Escaba, in southern Tucumán Province. The same is observed in the animal body color, with variations ranging from black to brilliant orange. Holotype measurements in the original description of Hylton Scott (1962) are not coincident with the real measurements of the holotype shell deposited in FML. In the original description of the present species, Hylton Scott (1962) states that only one mucous gland is present in the dart sac complex, although she remarked that this must be reconfirmed. Based on the dissections of several specimens of *E. variegata*, I have shown the presence of two mucous glands inserting in the dart sac complex. The genitalia of *E. variegata* is similar to *E. hemiclausa* Parodiz, the main differences being the shape of the dart sac and the mucous glands.

Epiphragmophora villavilensis Parodiz, 1955
(Figs. 6D, 17A, B, 21)

Epiphragmophora villavilensis Parodiz, 1955: 94, fig. 1.
Epiphragmophora villavilensis – Fernández & Rumi, 1984b: 253.

Holotype and Paratypes: MACN 383, Argentina, Catamarca, Dept. Andalgalá, Villavil 1933, M. Gomez leg.

Other Material Examined:

Dissected Material: Argentina, Catamarca Prov.: FML 14442 A, Belén Dept., Villavil, Quebrada de Indalecio, 27°05’19"S, 66°49’28"W, 2,220 m, C. Ituarte leg.

Dry Material: Argentina, Catamarca Prov.: MLP unnumbered, Termas de Hualfin. Ex Hylton Scott Collection. MLP unnumbered, Termas de Villavil, Cisneros leg. V/1950. MLP 4276, Termas de Villavil. MACN 383, Baños de Villavil. FML 14718, Belén Dept., Villavil, Quebrada de Indalecio, 27°05’19"S, 66°49’28"W, 2,220 m, 25/XI/2003, M. G. Cuezzo leg.

Description:

Shell (Fig. 6D): Dextral, helicoidal, depressed, semisolid; spire highly depressed; with 4½ whorls; body whorl periphery with supra-equatorial angle delimiting dorsal and ventral regions; periostracum pale yellow brilliant; peripheral pigmented band absent; protoconch smooth; teleochonch with fine axial growth lines, in body whorl more spaced with spiral stria; aperture subquadrate with reflexed thin, peristome; body whorl not descending behind aperture; umbilicus deep, wide.

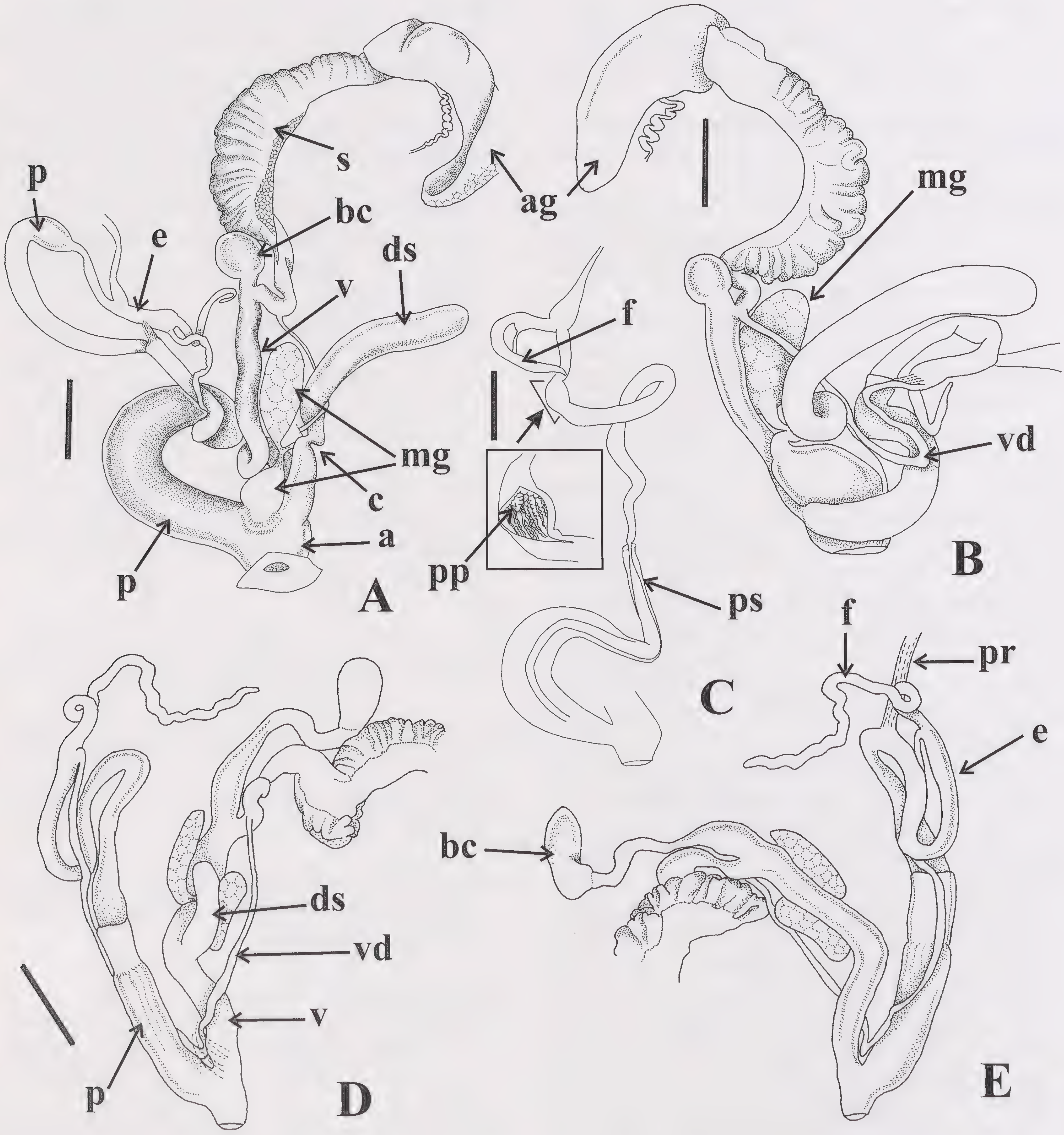


FIG. 14. *Epiphragmophora trenquelleonis* and *E. tucumanensis*. A: *E. trenquelleonis*, genital system in ventral view; B: *E. trenquelleonis*, genital system in dorsal view, different specimen; C: *E. trenquelleonis*, penial complex in stretched position, inset: detail of dissected proximal portion of penis showing penial papilla and pilasters of penial wall; D: *E. tucumanensis*, ventral view of terminal genitalia; E: *E. tucumanensis*, dorsal view of genitalia, same specimen. Abbreviations: a, atrium; ag, albumen gland; bc, bursa copulatrix; c, constriction; ds, dart sac; e, epiphallus; f, flagellum; mg, mucous gland; p, penis; pp, penial papilla; pr, penial retractor muscle; ps, penis sheath; s, spermoviduct; v, vagina; vd, vas deferens.

Measurements: Holotype: D maj: 27.2 mm; D min: 23.0 mm; H: 11.5 mm; D ap: 14.9 mm. Paratypes (n = 3): D maj: 21.1–28.9 mm (24.9 mm); D min: 18.3–24.7 mm (21.4 mm); H: 8.6–11.0 mm (9.5 mm).

Specimens from Type Locality (n = 8): D maj: 25.0–22.3 mm (23.5 mm); D min: 21.0–18.6 mm (19.5 mm); H: 10.0–8.6 mm (9.5 mm); D ap: 13.4–12.0 mm (12.4 mm); H ap: 11.3–9.5 mm (10.1 mm).

Genitalia (Fig. 17A, B): Terminal genitalia with dart sac complex consisting of single dart sac and two unequal dart glands; one of mucous glands sac-like, prominent, enveloping basal vagina and dart sac complex, other gland oval-elongate; efferent ducts of both mucous glands fused together before entering dart sac at constriction point; dart sac short, cylindrical, with thick muscular walls and marked constriction in medial zone; thin, internal dart gland in distal portion of dart sac; dart sac ending at atrium level with vagina and penis; bursa copulatrix round, with thick, short duct; vagina short, thick, almost completely covered by mucous gland; penial complex short; flagellum thin, finger-like short, ending level where vas deferens enters penial complex and epiphallus begins; epiphallus proximally thick, progressively thinner towards penis; epiphallus shorter than penis, about half its length; penial retractor thick, short, inserting in middle zone of epiphallus; penis cylindrical gradually becoming thinner until entering at genital atrium; penial papillae short, triangular in proximal penis; internal wall of penis with thin delicate longitudinal folds; penis sheath thin, overlapping terminal portion of penis, less than half length of penis; atrium very long.

Habitat and Distribution (Fig. 21): *Epiphragmophora villavilensis* inhabits the Pre-Puna biogeographical province of southern Catamarca Province, Argentina. Specimens are found in xerophilic habitats under rocks or in deep rock crevices. Although empty shells are easy to find on rocks or on the soil close to crevices, live specimens are very difficult to find. They live most of the day deep inside narrow crevices and are probably active only during part of the night or during rainy days.

Remarks: *Epiphragmophora villavilensis* differs from *E. hieronymi* in shell sculpture, absence of a peripheral band and the contour of body whorl (Table 1). The genitalia of *E. villavilensis* are similar to *E. hieronymi*, differing mainly in characters of penial complex, which is shorter in *E. villavilensis* than in the other species. The epiphallus is considerably shorter and the flagellum is longer in *E. villavilensis*. The point of insertion of the fused efferent ducts of the mucous glands is in the constriction of the dart sac, whereas in *E. hieronymi* the insertion point is below the constriction in the distal zone of the dart sac.

The description of the terminal genitalia is based on a single specimen collected by C. Ituarte at the type locality.

Epiphragmophora walshi Cuezzo, n. sp.

(Figs. 7C, 17C, D)

Holotype: FML 14765 A, Argentina, Salta, Metán Dept., route 5 road from Lumbrera to Las Víboras, 750 m, 29/XII/1996. M. G. Cuezzo leg.

Paratypes: FML 14764, Argentina, Salta, Metán Dept., Route 5 from Lumbrera to Las Víboras, 25°11’38"S, 64°53’07"W, 760 m, 27/IV/2005, leg. by E. Dominguez & M. G. Cuezzo. FML 14753 A, Metán Dept., Rout 5, Lumbrera. 29/XII/1996. M. G. Cuezzo leg.

Other Material Examined:

Dissected Material: Argentina, Salta Prov.: MLP unnumbered, Gral. José de San Martín Dept., Gral. Ballivián, 3/XII/1943. Maldonado leg. MLP unnumbered, Rivadavia Dept., La Curva, 1951, VII/1951, Boero leg.

Dry Material: Argentina, Salta: FML 809, Metán Dept., Estancia Lumbrera, 750 m leg. & det. W. Weyrauch. FML 577, Metán Dept., between Lumbreras and Juramento, km 1362, 750 m 23/II/1962. leg. & det. by W. Weyrauch. FML 14747, Metán Dept., route 5 to Lumbrera, XI/1996, M. G. Cuezzo leg. FML 389, Güemes Dept., Cerro Pelado, 1,500 m, 4/I/1956, E. de La Sota leg. FML 14763, Metán Dept., Route 9, 40 km N from Route 5, 24°53’40"S, 64°59’10"W, 900 m, 29/IV/2005, leg. M. G. Cuezzo.

Diagnosis: Shell medium to small; three thin continuous peripheral bands well marked in body whorl; aperture subcircular to oval; dart sac ending at atrium level with vagina and penial complex; proximal portion of dart sac wider than distal; two unequal mucous glands, one oval elongated in shape with a basal expansion; both mucous glands efferent ducts joining and fusing together before inserting in dart sac; epiphallus as long as penis.

Etymology: This new species is dedicated to Rodolfo Walsh, an Argentinean writer and journalist, with admiration for his work and ideas.

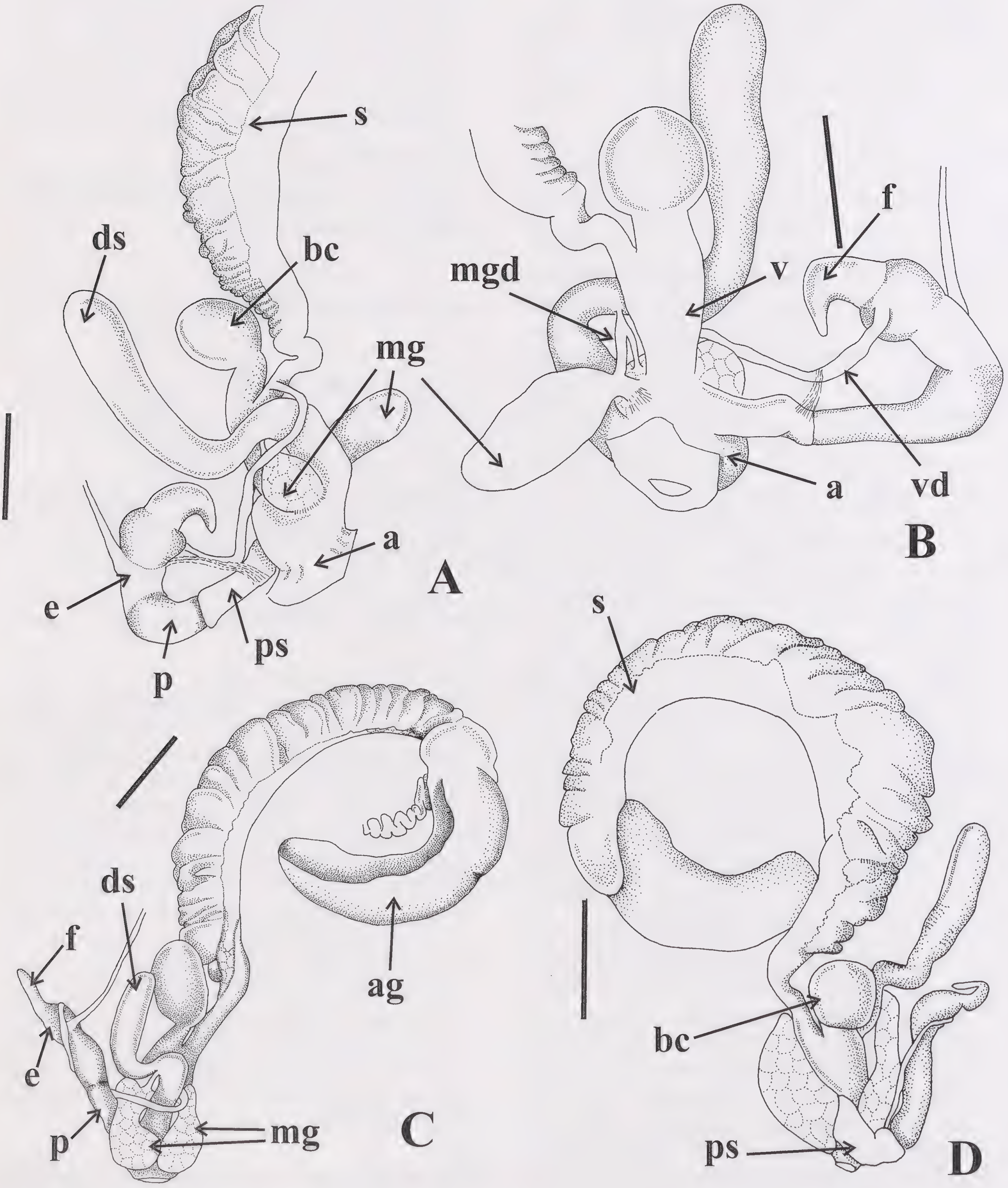


FIG. 15. *Epiphragmophora trifasciata* and *E. trigrammephora*. A: *E. trifasciata*, ventral view of terminal genitalia; B: *E. trifasciata*, dorsal view of terminal genitalia; C: *E. trigrammephora*, ventral view of genitalia; D: *E. trigrammephora*, other specimen showing genitalia in dorsal view. Scale bars = 5 mm. Abbreviations: a, atrium; ag, albumen gland; bc, bursa copulatrix; ds, dart sac; e, epiphallus; f, flagellum; mg, mucous gland; mgd, mucous gland ducts; p, penis; ps, penis sheath; s, spermoviduct; v, vagina; vd vas deferens.

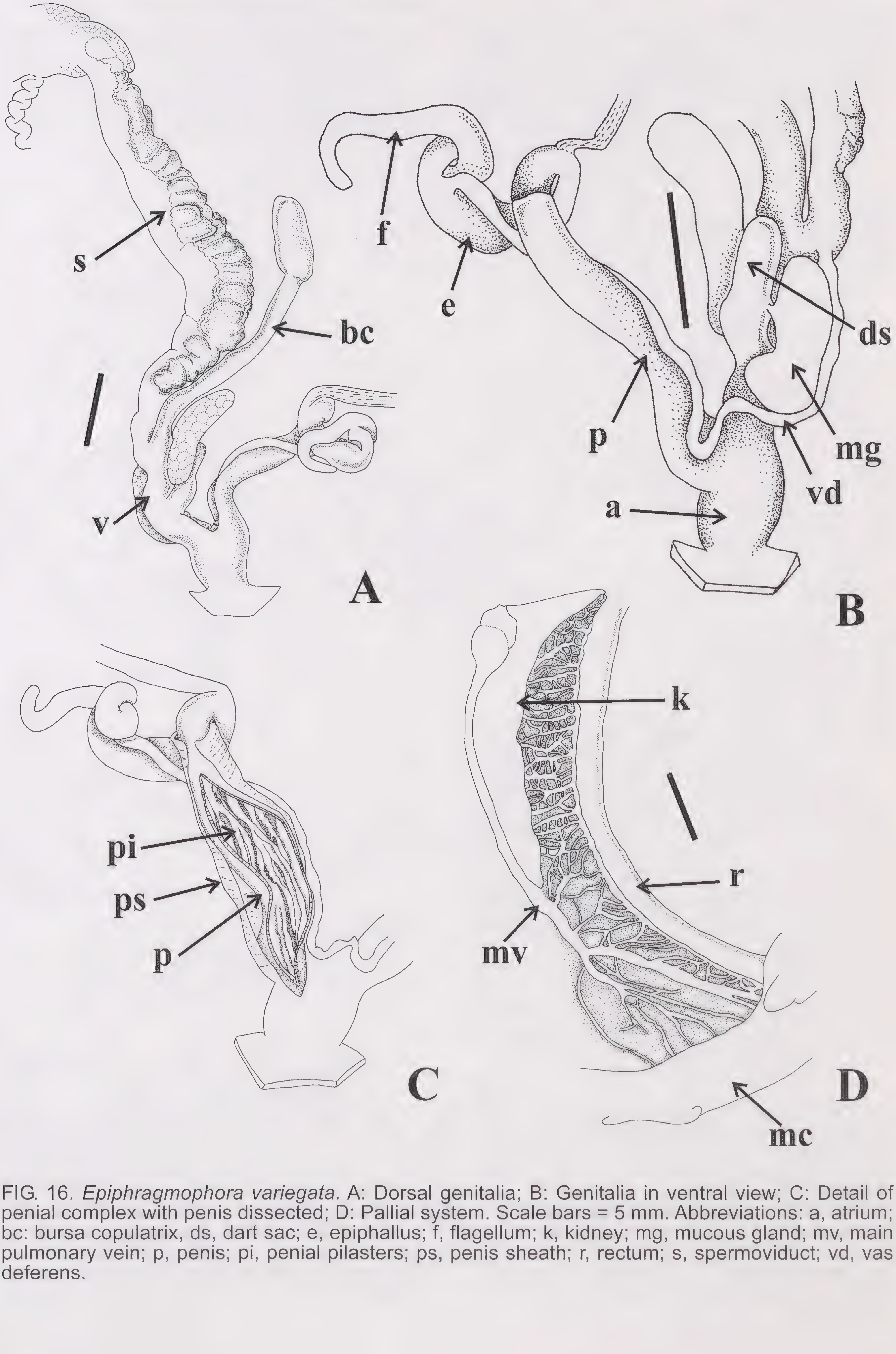
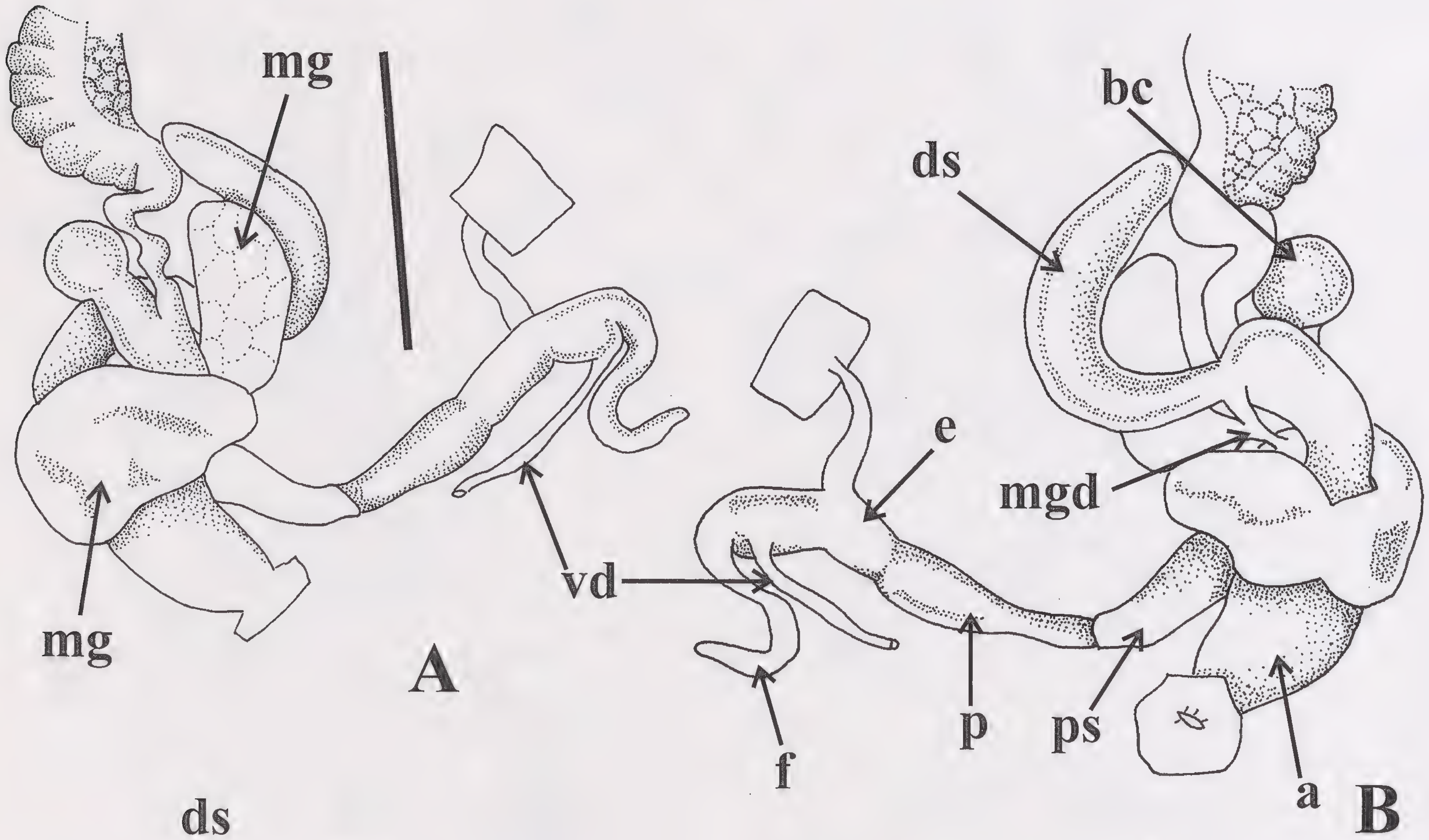


FIG. 16. *Epiphragmophora variegata.* A: Dorsal genitalia; B: Genitalia in ventral view; C: Detail of penial complex with penis dissected; D: Pallial system. Scale bars = 5 mm. Abbreviations: a, atrium; bc: bursa copulatrix, ds, dart sac; e, epiphallus; f, flagellum; k, kidney; mg, mucous gland; mv, main pulmonary vein; p, penis; pi, penial pilasters; ps, penis sheath; r, rectum; s, spermoviduct; vd, vas deferens.

Description:

External Features: Body color homogeneous light gray; some specimens with dorsal portion of cephalic region darker than foot color; single dorsal row of pustules from mantle collar to ommatophores well delimited.

Shell (Fig. 7C): Dextral, helicoidal, subglobose, semisolid; usually with low conical spire; medium to small size; 4¾ to 5¾ convex whorls; pale cream to yellow; three peripheral thin, darker brown bands clearly delimited on body whorl; upper band running



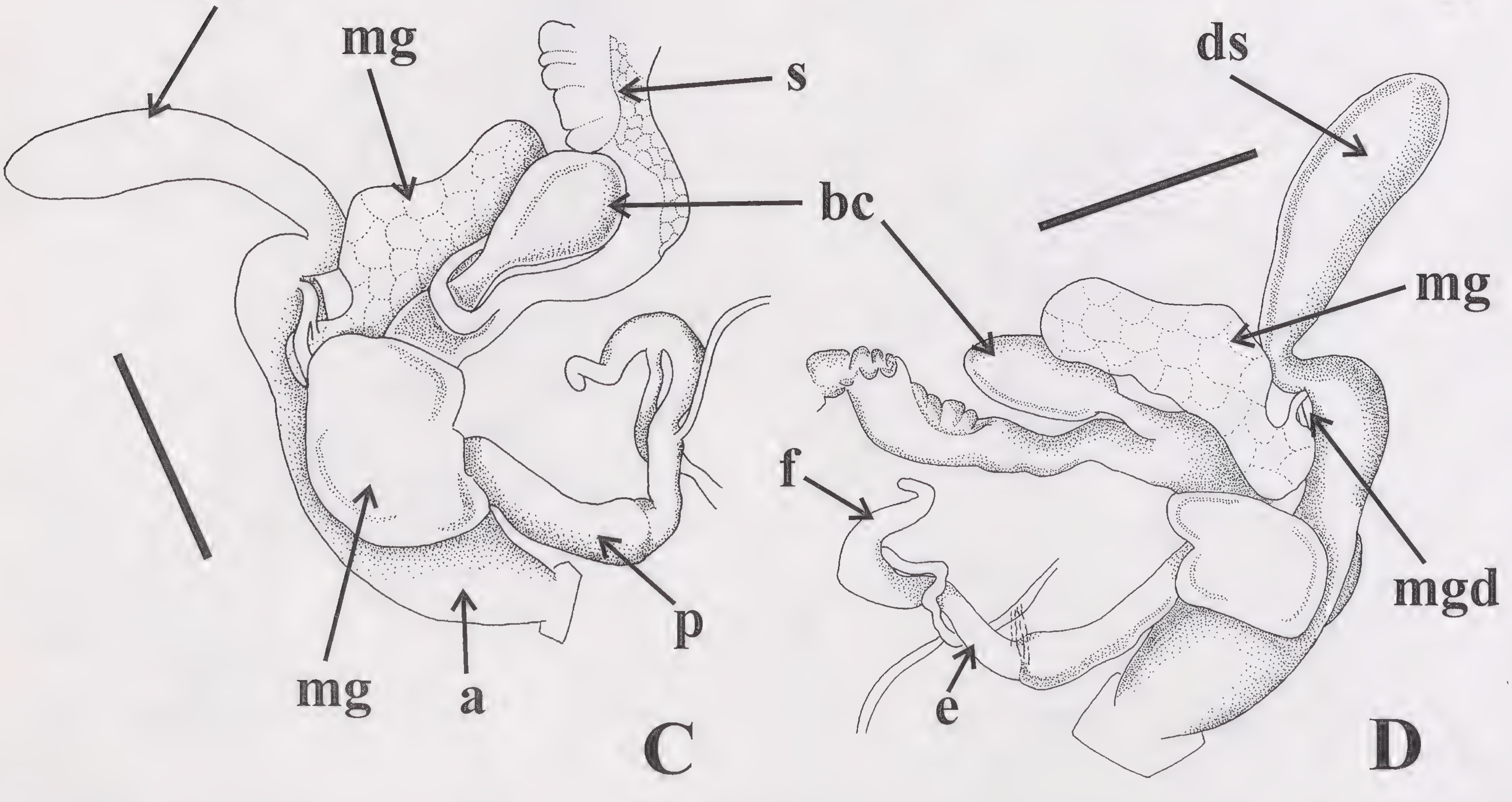


FIG. 17. *Epiphragmophora villavilensis* and ***E. walshi***. A: *E. villavilensis*, dorsal view of terminal genitalia; B: *E. villavilensis*, ventral view of terminal genitalia; C: ***E. walshi***, dorsal view of terminal genitalia; D: ***E. walshi***, ventral view of terminal genitalia. Scale bars = 5 mm. Abbreviations: a, atrium; bc, bursa copulatrix; ds, dart sac; e, epiphallus; f, flagellum; mg, mucous gland; mgd, mucous gland ducts; p, penis; ps, penis sheath; s, spermoviduct; vd vas deferens.

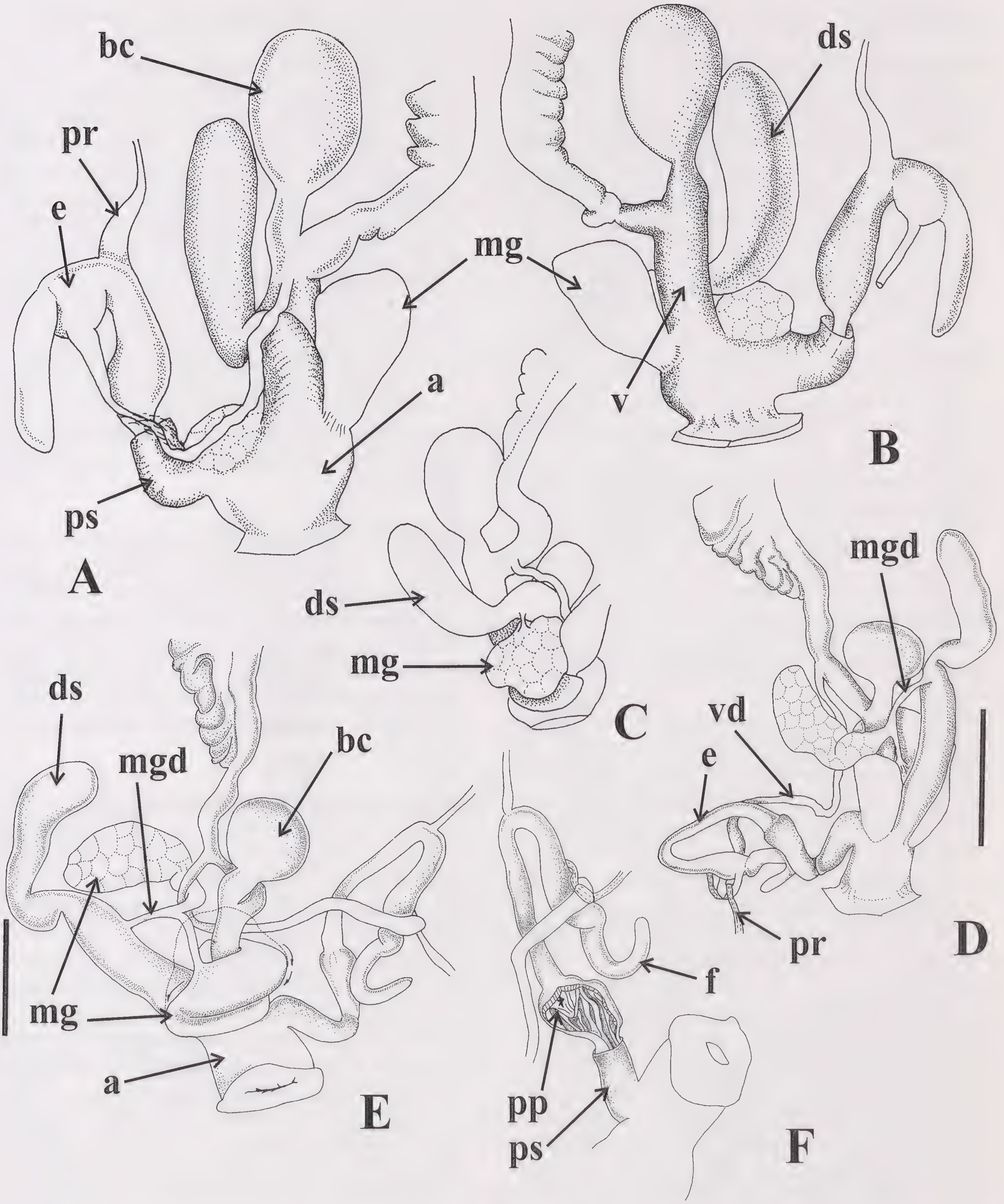


FIG. 18. ***Epiphragmophora guevarai*** and ***E. quirogai***. A: ***E. guevarai***, ventral view of terminal genitalia; B: ***E. guevarai***, dorsal view of terminal genitalia; C: ***E. guevarai***, lateral view of terminal genitalia showing point of insertion of mucous gland; D: ***E. quirogai***, dorsal view of terminal genitalia; E: ***E. quirogai***, ventral view of terminal genitalia; F: ***E. quirogai***, detail of penial complex with penis dissected. Scale bars = 5 mm. Abbreviations: a, atrium; bc: bursa copulatrix, ds, dart sac; e, epiphallus; f, flagellum; mg, mucous gland; mgd, mucous gland duct; p, penis; pp, penial papillae; ps, penis sheath; v, vagina; vd, vas deferens.

above suture in each anfractum of spire; bands always thin, with well-defined limits, sometimes central band darker; protoconch smooth, first whorls with thin growth lines; teleoconch surface generally malleated, with diagonal ribs and transverse growth lines interrupted by axial ribs on body whorl; suture deep impressed; aperture subcircular to oval, some specimens with basal lip straight, not curved; peristome white, thin, delicate, expanded, slightly reflexed; body whorl slightly descending towards aperture; umbilicus narrow, deep.

Measurements: Paratypes (n = 17): D maj: 20.0–25.5 mm (22.7 mm); D min: 16.8–22.4 mm (19.7 mm); H: 10.0–12.4 mm (11.4 mm); D ap: 10.6–14.3 mm (12.4 mm) H ap: 9.8–12.4 mm (11.1 mm).

Specimens from Type Locality (n = 10): D maj: 20.6–23.4 mm (22.7 mm); D min: 19.2–20.5 mm (19.4 mm); H: 11.4–13.3 mm (12.2 mm); D ap: 11.2–12.9 mm (12.3 mm); H ap: 9.9–12.5 mm (11.2 mm).

Jaw: Arched, thin, translucent; orange, with six vertical narrow ribs in central portion; upper and lower margins of ribs projecting at convex jaw edge.

Pallial Complex: Lung roof homogeneously pale cream; shell peripheral bands not marked on lung roof or mantle collar; kidney extending 60% of total pulmonary roof length; main pulmonary vein splitting proximally in two mayor veins that further divide into minor veins before reaching mantle collar; portion of lung roof between rectum and kidney crossed by several minor transversal ramified veins giving appearance of complex net.

Genitalia (Fig. 17C, D): Dart sac complex in terminal genitalia consisting of single dart sac and two unequal mucous glands; one mucous gland sac-like, partially enveloping distal vagina and dart sac, distally fused with atrium wall, with single thin efferent duct; other mucous gland elongate-oval, with basal globose expansion from which efferent duct departs; both efferent ducts joining and fusing together before inserting in dart sac; dart sac muscular, with thick walls, cylindrical, with a median constriction; proximal portion of dart sac wider than distal portion; vas deferens surrounding dart sac level with constriction before inserting in penial complex; dart papillae half length of distal portion of dart sac; dart sac ending at atrium level with vagina and penial complex; bursa copulatrix sac round to oval, with very short duct; sac and duct of bursa copulatrix neatly delimited; vagina shorter than penis; free oviduct short, enveloped by connective tissue together with bursa copulatrix; penial complex medium to short in total length; flagellum thin, finger-like, gradually tapering towards tip, ending level where vas deferens enters penial complex and epiphallus begins; proximal portion of epiphallus widens at point of entrance of vas deferens; epiphallus as long as penis or slightly longer; penial retractor muscle inserting in median portion of epiphallus; penis cylindrical, with proximal portion slightly wider, entering at genital atrium level with dart sac and vagina; penial papillae short, triangular in proximal penis; internal wall of penis with thin smooth zigzag pleats; penial sheath muscular, gradually becoming thinner towards proximal penis, distally fused to atrium wall; atrium long, thin.

Habitat and Distribution: In xerophilic environments where species of *Naesiotus* and *Bulimulus* (Bulimulidae) are also abundant. Live specimens are found inside shallow holes in the ground or attached to basal portions of shrubs. ***Epiphragmophora walshi*** is distributed in the Chacoan biogeographic subregion in Salta Province, northwestern Argentina.

Remarks: ***Epiphragmophora walshi*** n. sp. is similar to *E. trigrammephora* in shell shape and in having three peripheral pigmented bands on the body whorl. It differs from *E. trigrammephora* in its smaller shell diameter, and in the apertural shape, which is subcircular or oval instead of subquadrangular. The peripheral bands on the body whorl in ***E. walshi*** are always very thin and well delimited. Main differences in the genitalia between both mentioned species are: (1) the shape and position of one of the mucous glands, which in ***E. walshi*** is oval-elongate, with a basal expansion, whereas in *E. trigrammephora* both mucous glands are sac-like enveloping basal vagina and dart sac; (2) the length of the epiphallus, which is longer in ***E. walshi*** than in *E. trigrammephora*. Differences in the number of vertical ribs in the jaw of both species are also found, with ***E. walshi*** having six ribs while *E. trigrammephora* has only four vertical central ribs. ***Epiphragmophora walshi*** has been found exclusively in xerophilic environments of Salta Province, whereas *E. trigrammephora* also inhabits humid forests from Bolivia to Salta.

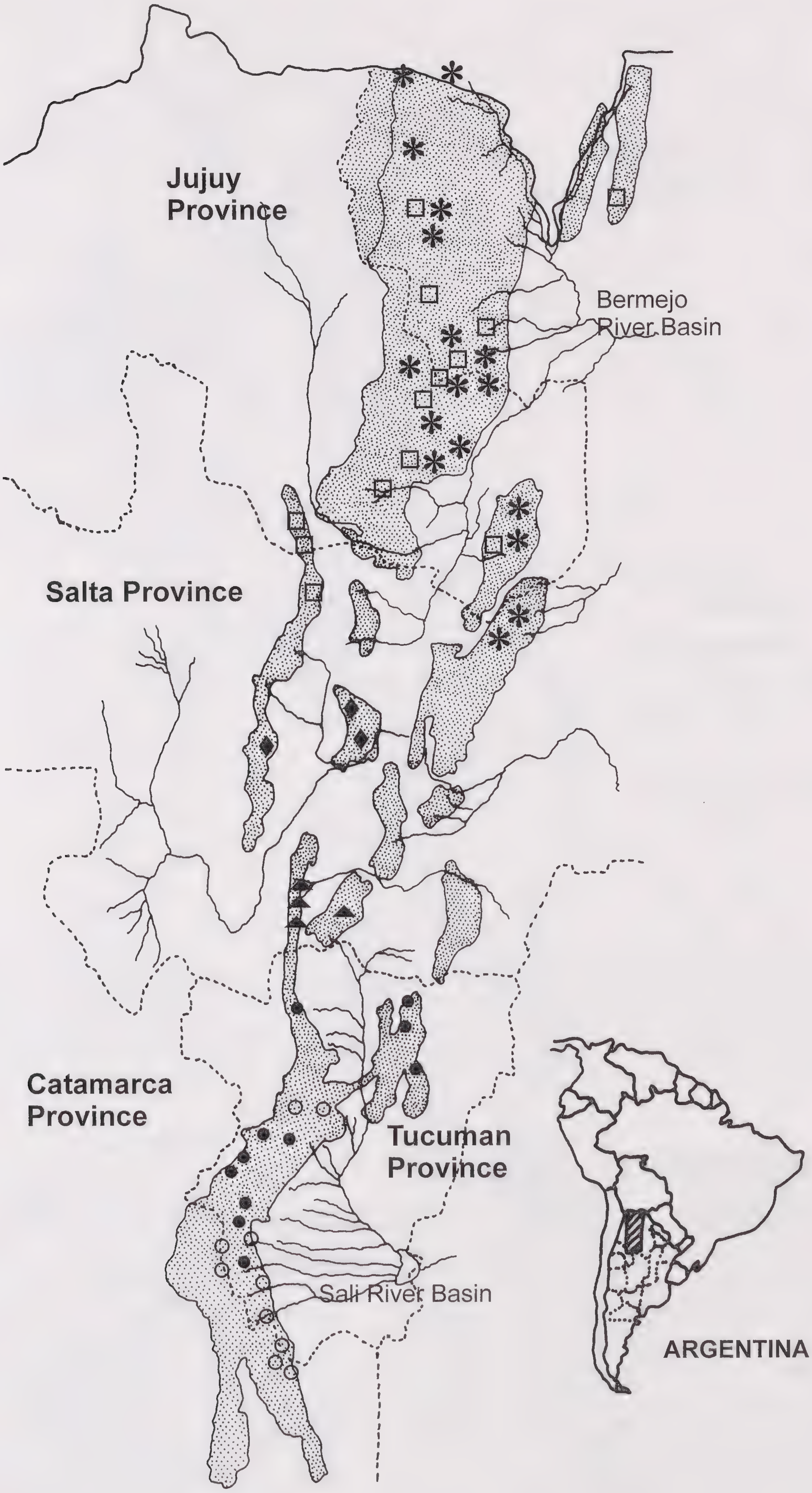


FIG. 19. Species distributed in the cloud rainforest biogeographic province of the Yungas: ● = *E. tucumanensis*; ▲ = *E. saltana*; ✱ = *E. jujuyensis*; O = *E. hemiclausa*; ☐ = *E. trigrammephora*; ◆ = *E. escoipensis*. Spotted areas correspond to the Yungas biogeographic province. Dot lines indicate the political limits of the argentinean provinces.

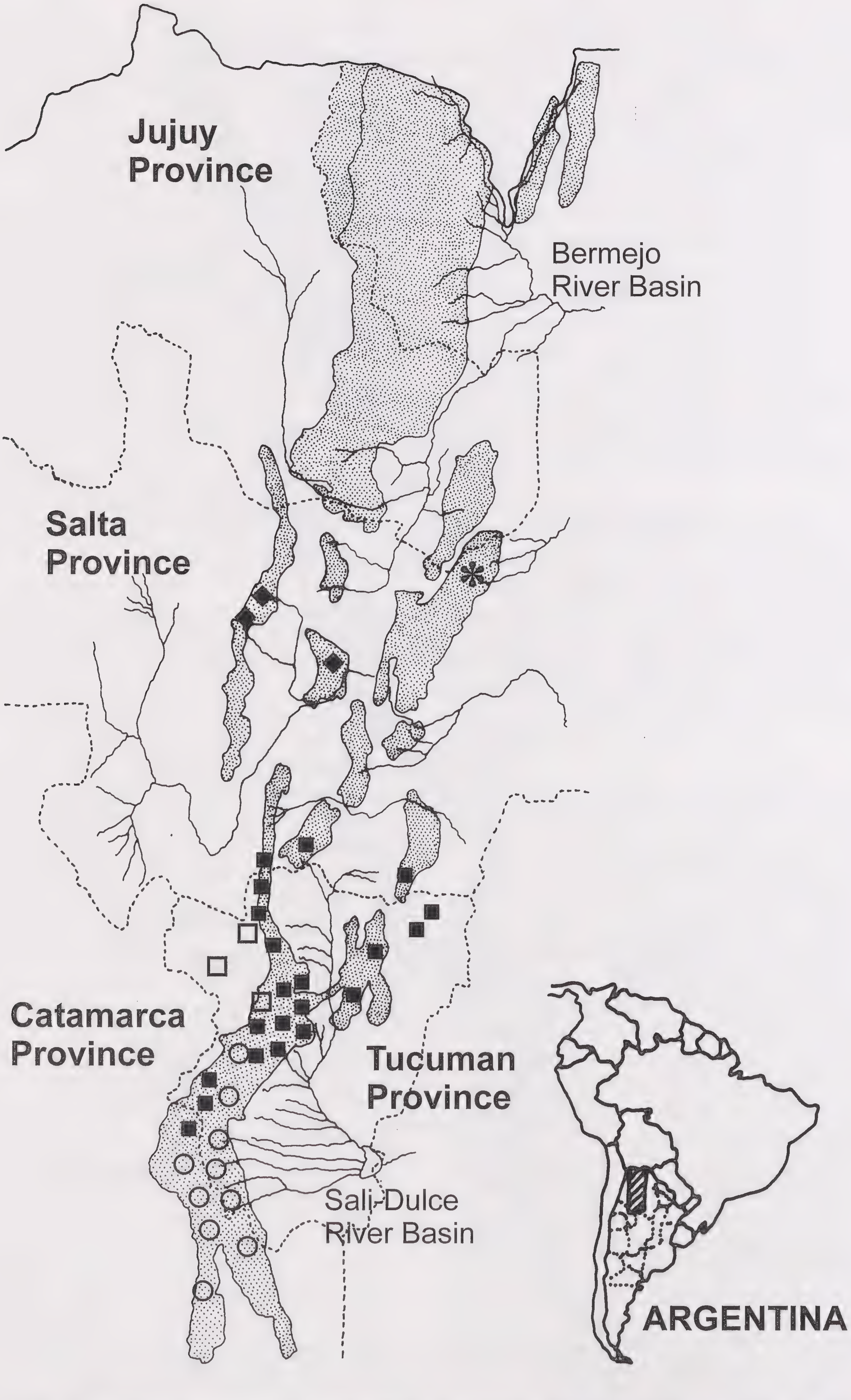


FIG. 20. Species ditributed in the cloud rainforest biogeographic province of the Yungas: O = *E. variegata*; ■ = *E. argentina*; ✱ = *E. costellata*; □ = *E. parodizi*; ◆ = *E. rhathymos.*

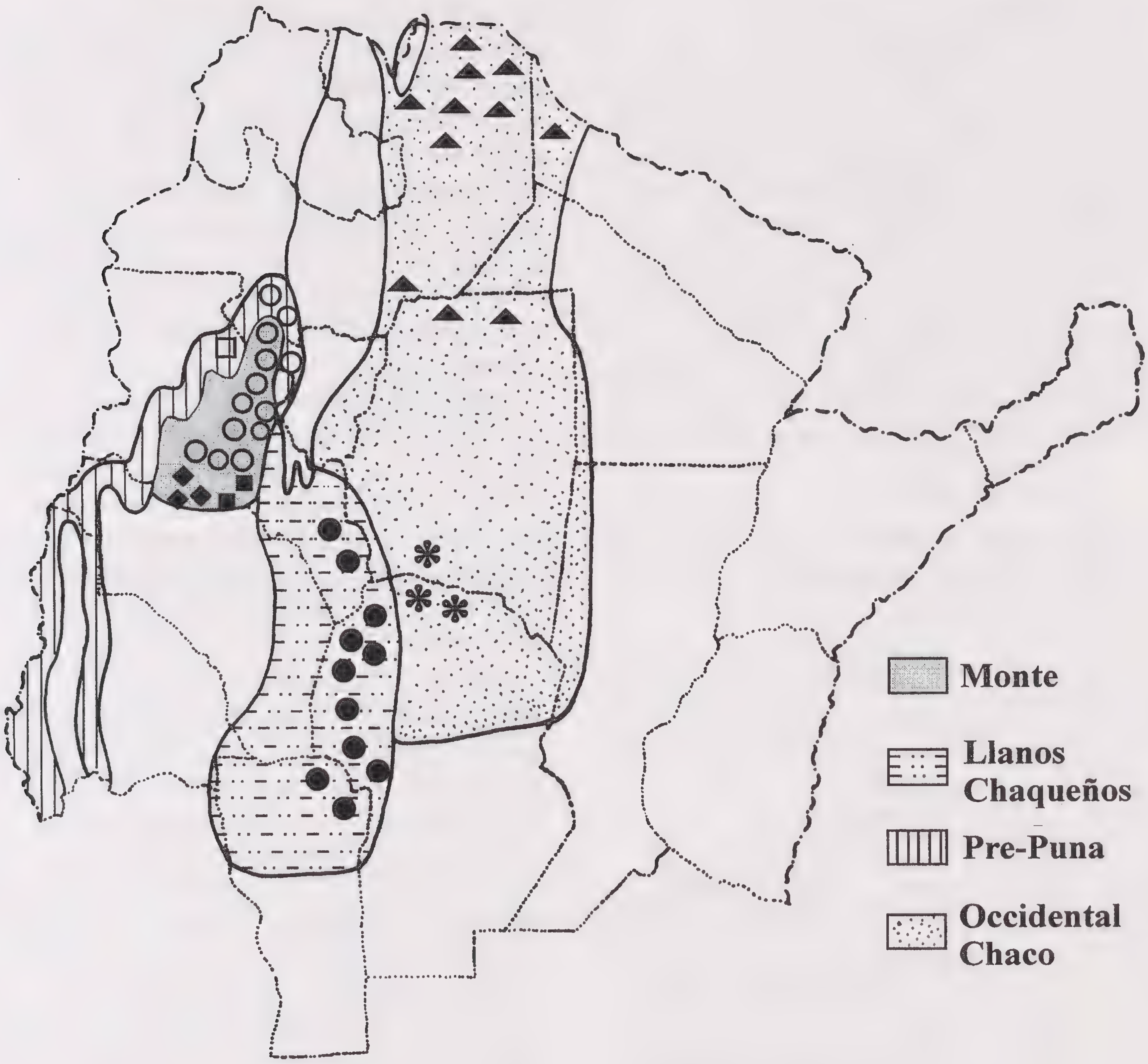


FIG. 21. Species distributed in the Chaco (Llanos Chaqueños and Chaco Occidental areas of endemism), Monte and Pre-Puna biogeographic provinces. Dot lines indicate the political limits of the argentinean provinces. Scale bar = 100 km. ▲ = *E. trigrammephora*; ■ = *E. puella*; ◆ = ***E. quirogai***; o = *E. hieronymi*; ✱ = ***E. guevarai***; ● = *E. trenquelleonis* and □ = *E. villavilensis.*

Parsimony Analysis

All the species treated were considered terminals and analyzed using *Bradybaena* to root the trees. In this first analysis, nine optimal trees with a fit of 190.7 were found. The strict consensus tree of these fundamental trees is illustrated in Fig. 22. Six unambiguous synapomorphies support the monophyly of the genus, with a good Bremer score of 6.0: body whorl surface malleated with diagonal ribs (character 1 [2]), umbilicus overlapping but not fused to body whorl (character 2 [1]), peristome thick, widely reflexed (character 4[1]), mucous glands unequal in size and shape (character 15 [1]), insertion of mucous glands ducts in middle portion of dart sac (character 17 [1]), penial retractor muscle inserting in medial epiphallus (character 23 [1]).

Epiphragmophora argentina, E. hemiclausa, E. tucumanensis, E. variegata form a clade in the majority of the nine optimal trees obtained; *E. jujuyensis* and *E. oresigena* were a sister group. However, three taxa, *E. audouini, E. oresigena* and *E. estella*, were unstable, being located in different trees as sister group of *E. jujuyensis* or alternatively forming part of the first-mentioned clade. Consequently, these clades collapsed in the strict consensus tree (Fig. 22). A major subgroup supported in the strict consensus tree is formed by ten *Epiphragmophora* species. The supporting synapomorphies of this main clade are: peristome slightly reflexed (character 4 [0], spire shallow conic (character 5 [1]), right mucous gland distally fused with atrial wall (character 20 [1]), penial papillae present (character 22 [1]), duct of bursa copulatrix extremelly short

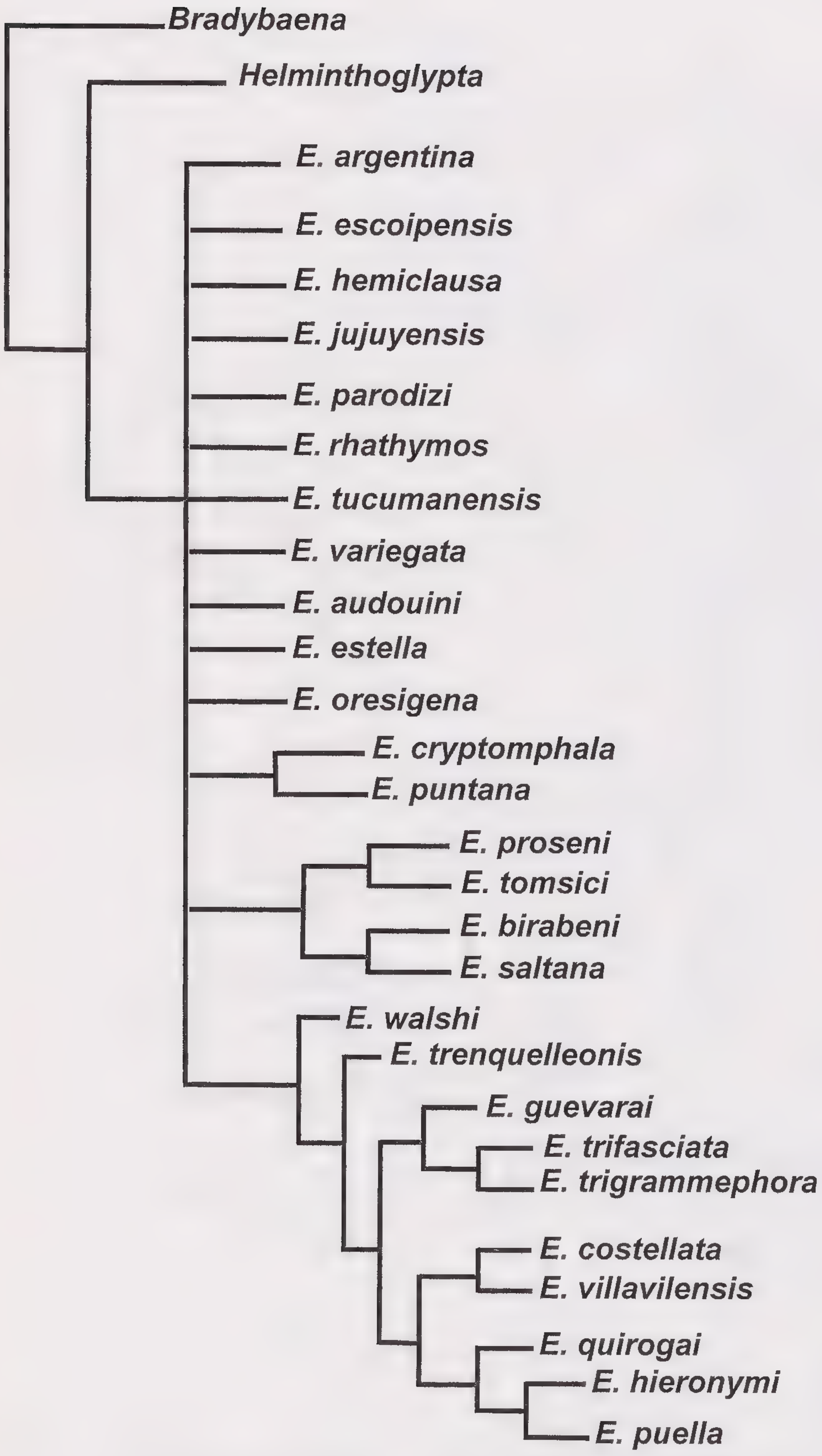


FIG. 22. Strict consensus tree of nine optimal trees obtained with PeeWee and illustrated with Winclada (first cladistic analysis).

(character 24 [0]), atrium medium to long, not expanded into an atrial sac (character 27 [1]), habitat preferences associated with rocks (character 31 [0]), and epiphallus proximal portion widening at point of entrance of vas deferens (character 33 [1]).

This main clade is subdivided into two main groups in the strict consensus tree with good resolution of the relationships and some unambiguous synapomorphies, but each has a low Bremer support (0.4). Within the second subgroup, character 23 [2] supports the monophyly of the following group: ((*E. hieronymi, E. puella*) ***E. quirogai*** n. sp.). Another clade with good support is formed by *E. costellata* and *E. villavilensis*, sustained by the unambiguous synapomorphies of characters 5 [2] spire height strongly depressed and character 8 [2] body-whorl periphery supraequatorally subcarinated. ((*E. trifasciata, E. trigrammephora*) *E. guevarai*)) conform a basal sister clade of the above-mentioned species. Two smaller clades are also mantained in the strict consensus tree, the sister species *E. cryptomphala* and *E. puntana* and ((*E. proseni*, *E. tomsici*) (*E. birabeni, E. saltana*)). These two clades show no resolution on their relationships respect to the other previously mentioned clades.

In the second analysis, all species known only by their shell characters were eliminated from the matrix for two reasons: (1) because some of them showed to be highly unstable, jumping from a clade to another in the different hypothesis obtained, as was explained in the case of *E. audouini* and *E. oresigena*; (2) to see if the relationships of the species with more characters improved with the elimination of those species known only by a part of their bodies. Consequently, *E. birabeni, E. costellata, E. proseni, E. audouini, E. estella* and *E. oresigena* were deleted from the original matrix.

This second analysis produced only one tree with a fit of 187.5 illustrated in Figure 23. In this tree, the genus is well supported by the following unambiguous synapomorphies, characters 1 [2], 2 (1), 4(1), 15 (1), 17 (1), 23 (1), which are also present in the first analysis. Two main clades are delimited, the first one (node 25) with a Bremer support score of 2.0 is: ((*E. argentina, E. tucumanensis*) *E. hemiclausa*) *E. variegata*) supported by character 26 (1). The second clade (node 41), with a good Bremer support score (3.0) is formed by the rest of the *Epiphragmophora* species, the monophyly of which is supported by the following synapomorphies: dart sac long, finger-like with a constriction (character 12 [0]), dart sac inserted in atrium (character 13 [1]), and duct of bursa copulatrix of medium length (character 24 [1]). Within this second main clade (node 41), E. *jujuyensis* is sister group of the rest of the species components that form a big sister clade (node 40). The monophyly of the clade in node 40 is supported by the following synapomorphies: 5 [1], 16[1], 19[1], 21[1] and 32[0]. Within the present clade (node 29), *E. villavilensis* is sister group of ((*E. hieronymi* (*E. puella*, ***E. quirogai*** n. sp.)) and together are sister groups of (node 32) *E. trifasciata*, ***E. guevarai*** n. sp. and *E. trigrammephora.*

Shell characters showed a better fit in the tree obtained in the second analysis with only characters 1, 3 and 7, with fit below 5.0, while in the first analysis 6 out of 10 characters showed a fit below 5.0. Their extra steps are also notably lower. Anatomical characters mostly maintained their good fit in comparison to the consensus tree of the first analysis (Table 2). If we consider the stability of the taxa, the better Bremer support scores obtained for the clades and the better resolution of the relationships, the tree obtained in the second analysis is preferred as the phylogenetic hypothesis over the consensus tree obtained in the first analysis.

DISCUSSION

The comparative anatomy of the treated species revealed that the morphology of the dart sac complex (= stimulator by other authors) is unique and informative in assesing the phylogenetic relationships among the different species of the genus. In *Epiphragmophora,* the insertion of the dart sac is either in the atrium or in the vagina, and the insertion of the two unequal mucous glands is in the medial zone of a single dart sac. The position, as well as the shape of the mucous glands inserting in

TABLE 2. Character steps (ES), extra steps (ESi), minimun and maximun steps (Min/max) and weight implemented by PeeWee consensus tree of the first analysis (left collumn) (Fig. 22) and optimal tree of the second analysis (right collumn) (Fig. 23).

Character	ES	ESi	Min/max	Weight	Character	ES	ESi	Min/max	Weight
1	9	5	4/15	3.7	1	8	4	4/13	4.2
2	6	4	2/13	4.2	2	4	2	2/11	6.0
3	7	5	2/14	3.7	3	6	3	2/9	4.2
4	6	4	2/14	4.2	4	5	3	2/11	5.0
5	4	2	2/15	6.0	5	4	2	2/11	6.0
6	-	-	1/1	-	6	-	-	1/1	
7	11	9	2/14	2.5	7	7	5	2/10	3.7
8	2	0	2/3	10.0	8	-	-	2/2	-
9	3	2	1/4	6.0	9	3	2	1/4	6.0
10	9	7	2/11	3.0	10	5	3	2/6	5.0
11	-	-	1/1	-	11	-	-	1/1	-
12	2	0	2/6	10.0	12	2	0	2/6	10.0
13	2	0	2/6	10.0	13	2	0	2/6	10.0
14	-	-	1/1	-	14	-	-	1/1	-
15	1	0	1/2	10.0	15	1	0	1/2	10.0
16	2	1	1/6	7.5	16	2	1	1/6	7.5
17	1	0	1/2	10.0	17	1	0	1/2	10.0
18	4	3	1/6	5.0	18	4	3	1/6	5.0
19	2	1	1/10	7.5	19	2	1	1/10	7.5
20	1	0	1/9	10.0	20	1	0	1/9	10.0
21	11	9	2/16	2.5	21	11	9	2/16	2.5
22	3	2	1/10	6.0	22	3	2	1/10	6.0
23	4	2	2/6	6.0	23	4	2	2/6	6.0
24	4	2	2/15	6.0	24	4	2	2/15	6.0
25	-	-	1/1	-	25	-	-	1/1	
26	3	1	2/6	7.5	26	3	1	2/6	7.5
27	6	4	2/11	4.2	27	6	4	2/11	4.2
28	5	3	2/6	5.0	28	5	3	2/6	5.0
29	2	1	1/4	7.5	29	2	1	1/4	7.5
30	2	1	1/3	7.5	30	2	1	1/3	7.5
31	2	1	1/3	7.5	31	2	1	1/10	7.5
32	3	2	1/7	6.0	32	3	2	1/7	6.0
33	5	4	1/10	4.2	33	5	4	1/10	4.2
34	2	1	1/3	7.5	34	2	1	1/3	7.5
35	-	-	1/1	-	35	-	-	1/1	-

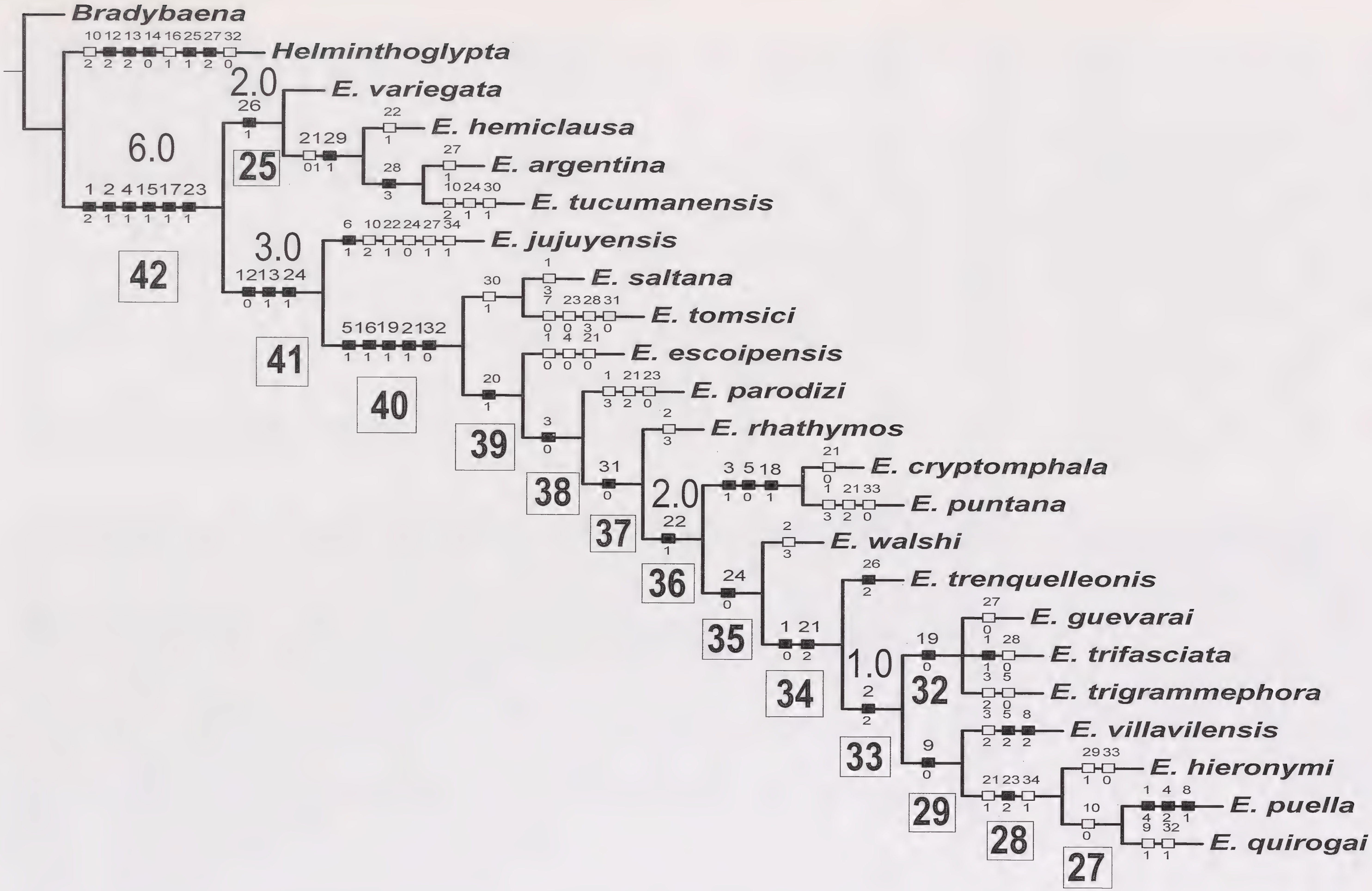


FIG. 23. Optimal tree obtained eliminating all taxa defined only by shell characters with no anatomical information from the matrix (second analysis). Black squares illustrating unambiguous synapomorphies according to PeeWee. Numbers below squares are state characters, Numbers above squares are characters detailed in Table 2. Bremer support scores are the numbers in bold above nodes. Big numbers within empty squares are node numbers.

the dart sac, are constant characters in all and each species studied. For this reason, the morphology of the terminal genitalia, especially referring to the dart sac complex, has been used to characterize each of the species treated.

Traditionally, some species such as *E. trenquelleonis, E. hieronymi* and *E. trigrammephora,* those with the widest areas of distribution that were mostly known only by their shells, have been suspected to be groups of species instead of single taxonomic units. In the present study, intensive fieldwork and collections of live specimens of *E. trenquelleonis* in different localities made possible the study on the variability of shell and anatomy, confirming its validity as a single species. *Epiphragmophora hieronymi* had been split into ***E. quirogai*** n. sp. and *E. hieronymi* species. Both species showed differences in shell morphology and were simpatric in some regions of Catamarca Province, northwestern Argentina. The analysis of the genitalia allows us to recognize the existence of two groups with a constant pattern of their genitalia. *Epiphragmophora trigrammephora* has also been exhaustively collected in localities from different ecoregions, including its type locality. Small specimens from Salta Province, usually identified as *E. trigrammephora* by shell characters, showed conspicuous differences also in the terminal genitalia so that a new species, ***E. walshi*** n. sp., has been erected.

The reconstructed phylogeny based on morphological data clearly supports the monophyly of *Epiphragmophora* on the base of six unambiguous synapomorphies and a good Bremer support score of 6.0. The cladistic hypothesis obtained is basally split into two main monophyletic sister clades, that also present a good Bremer support score (Fig. 23, node 42). Characters of the distal portion of the reproductive tract, such as the length of the vagina, shape and point of insertion of the dart sac complex, number and shape of mucous glands, are among the most informative characters. Shell characters proved to be less informative than anatomical characters (compare behavior of shell characters respect anatomical characters in Table 2). The species known only by shell characters, especially *E. audouini, E. estella* and *E. oresigena,* were unstable in their position in the cladograms, proposing relationships not well supported. The short duct of the bursa copulatrix, traditionally treated as an important character to diagnose the genus/subfamily by Pilsbry (1939), is not characteristic of all studied species but only from a small group of them (node 35, Fig. 23). The diverticulum of the bursa copulatrix, another character traditionally considered as a synapomorphy of Helicoidea, is absent in all studied species of *Epiphragmophora.* The diverticulum of the bursa copulatrix is present, however, in genera close to *Epiphragmophora* such as *Helminthoglypta.*

According to the preferred phylogenetic hypothesis of *Epiphragmophora* presented here (Fig. 23), the insertion of the dart sac (character 13) occurred in the vagina in one of the main monophyletic groups of species (*E. variegata, E. hemiclausa, E. argentina* and *E. tucumanensis*) whereas in its sister group (rest of the treated species, node 41) the insertion of the dart sac is in the atrium. In the first-mentioned clade, the dart sac does not have a medial constriction that is present in all the rest of the species. In the species of the second clade (node 41), one of the mucous glands is sac-like, surrounding the basal genitalia, usually being attached to the atrium wall. In the species of the first group (node 25), the mucous glands are usually oval to cylindrical, with efferent ducts not fused to each other.

Within the second clade, *E. villavilensis, E. hieronymi, E. puella* and ***E. quirogai*** n. sp., the smallest species of the genus in shell diameter are grouped into a derived monophyletic clade (node 29) with a high Bremer support score. This mentioned group of species is almost exclusively distributed in the Monte and Pre-Puna biogeographic provinces. *Epiphragmophora trifasciata, E. trigrammephora* and ***E. guevarai*** n. sp. also form a monophyletic clade (node 32), although the relationships among them are not well resolved.

Although the taxon sample used in the present hypothesis is not insignificant, as the genus is represented by more than the 50% of the known species, I do not propose subdivisions of *Epiphragmophora* until the anatomy of the northern species is known and a new cladistic analysis can be carried out. However, *E. hieronymi*, the type species of *Epiphragmophora* s.s., and *E. trenquelleonis*, the type species of *E.* (*Doeringina*), both belong to the second clade (node 41) delimited in the preferred tree (Fig. 23). Thus, the validity of these previously stablished subgenera as independent taxonomic identitites is dubious based on my preliminary phylogeny of the genus.

Among the Helicoidea, the dart sac complex (= stimulatory organ) generally inserts at the vagina except for the Sphincterochilidae, in which the insertion is at the atrium, and in some Helminthoglyptinae and Cepoliinae, in which the dart sac complex inserts at the atrial sac (= neophore). A "dart sheath" (Pilsbry, 1939), or a "basal genital sheath" (Cuezzo, 1998), present in Cepoliinae and Helminthoglyptinae, is absent in all *Epiphragmophora* species studied.

Nordsieck (1987) considered that the insertion of the dart sac complex at the atrium was an autapomorphy of the Helicoidea. Hausdorf (1998) stated that the insertion of the dart sac complex (= stimulator) on the atrium is not an autapomorphy but a symplesiomorphy shared with the Limacoidea *sensu lato.* He also supported the idea that in the ancestral state of the Cepoliidae + odontognath Helicoidea, the dart sac complex does not insert on the vagina but on the atrium, as in the Sphincterochilidae and in the Limacoidea *sensu lato.* Narrative hypotheses on the evolution of the dart sac complex established for the genus/subfamily until now (Ihering, 1929; Nordsieck, 1987; Schileyko, 1991) are not relevant, because they are not based on strict character analysis and testable methodologies. Further anatomical studies and new cladistic analyses in Helicoidea are necessary before a better understanding on the evolution of the dart sac complex is achieved.

Biogeographic Considerations

The species of *Epiphragmophora* that inhabit rainforest areas called Yungas – *E. tucumanensis, E. argentina, E. hemiclausa, E. variegata, E. jujuyensis,* and *E. saltana* – that are characteristics species living in relation to humids tree logs, occupy a basal position in the area cladogram (Fig. 24). Three species are widely distributed into the Yungas and Chacoan provinces – *E. trigrammephora, E. tomsici* and *E. rhathymos.* The rest of the *Epiphragmophora* species inhabit more arid environments of Chaco, Monte and Pre-Puna provinces and are found living in association to rocks, usually within narrow crevices. The Monte and Pre-Puna areas conform a monophyletic clade with a more derived position in the area cladogram (Fig. 24).

The Amazonian biogeographic subregion show closed relationships to the Chacoan biogeographic subregions. The development of the Chacoan subregion during the Tertiary splits the formerly continuous Amazonian forest into the Yungas (northwestern Argentina-southern Bolivia) and Paraná rainforest (northeastern Argentina-southern Brazil) representing an example of dynamic vicariance (Morrone, 2006). The Monte province comprises central Argentina between latitudes 24°S and 43°S, its fauna is basically of Chacoan origin, although some elements are Patagonian, Prepunean and Subantartic. It has been suggested that the Monte represents an "impoverished", Chaco (Morrone, 2006). A cladistic biogeographic analysis based on beetle and plant taxa showed that these provinces are closely related (Morrone, 1993). Roig Juñet & Flores (2001) divided the Monte province into three areas of endemism – the Boreal Monte, Central Monte, and Austral Monte. The Boreal Monte extends from Salta to northern La Rioja provinces through three longitudinal valleys limited by mountains higher than 3,000 m. ***Epiphragmophora quirogai*** sp. nov. and *E. puella* are endemic of the Boreal Monte area. No species of the genus have been collected or found in the other two areas of endemism of the Monte province. Within the Chacoan subregion two areas of endemism had been delimited (Roig Juñet & Flores, 2001), the Occidental Chaco and the "Llanos Chaqueños". The Occidental Chaco is an area that extends from southern Bolivia and western Paraguay to northern Córdoba Province in Argentina. *Epiphragmophora trigrammephora* and ***E. walshi*** inhabit this area of endemism. The "Llanos Chaqueños", ranges from southwestern Santiago del Estero Province through western part of Córdoba and eastern portion of Catamarca to northern San Luis Province. ***Epiphragmophora guevarai***, *E. trenquelleonis, E. puntana, E. tomsici* and *E. trifasciata*´s areas of distribution are coincident with the "Llanos Chaqueños", area of endemism. Some of the *Epiphragmophora* species are habitants of the Pre-Puna, an area recently classified as a "South American Transition zone" (Morrone, 2006). This transitional zone extends along the highlands of the Andes between western Venezuela, northern Chile and west-central Argentina. The Pre-Puna and Monte provinces, previously assigned to the Neotropical region, are actually assigned to the transitional region because of the closed affinity existing between the Puna and the North Andean Paramo (Morrone, 2002, 2006). Although the species richness of such invertebrates as molluscs and insects in the Pre-Puna

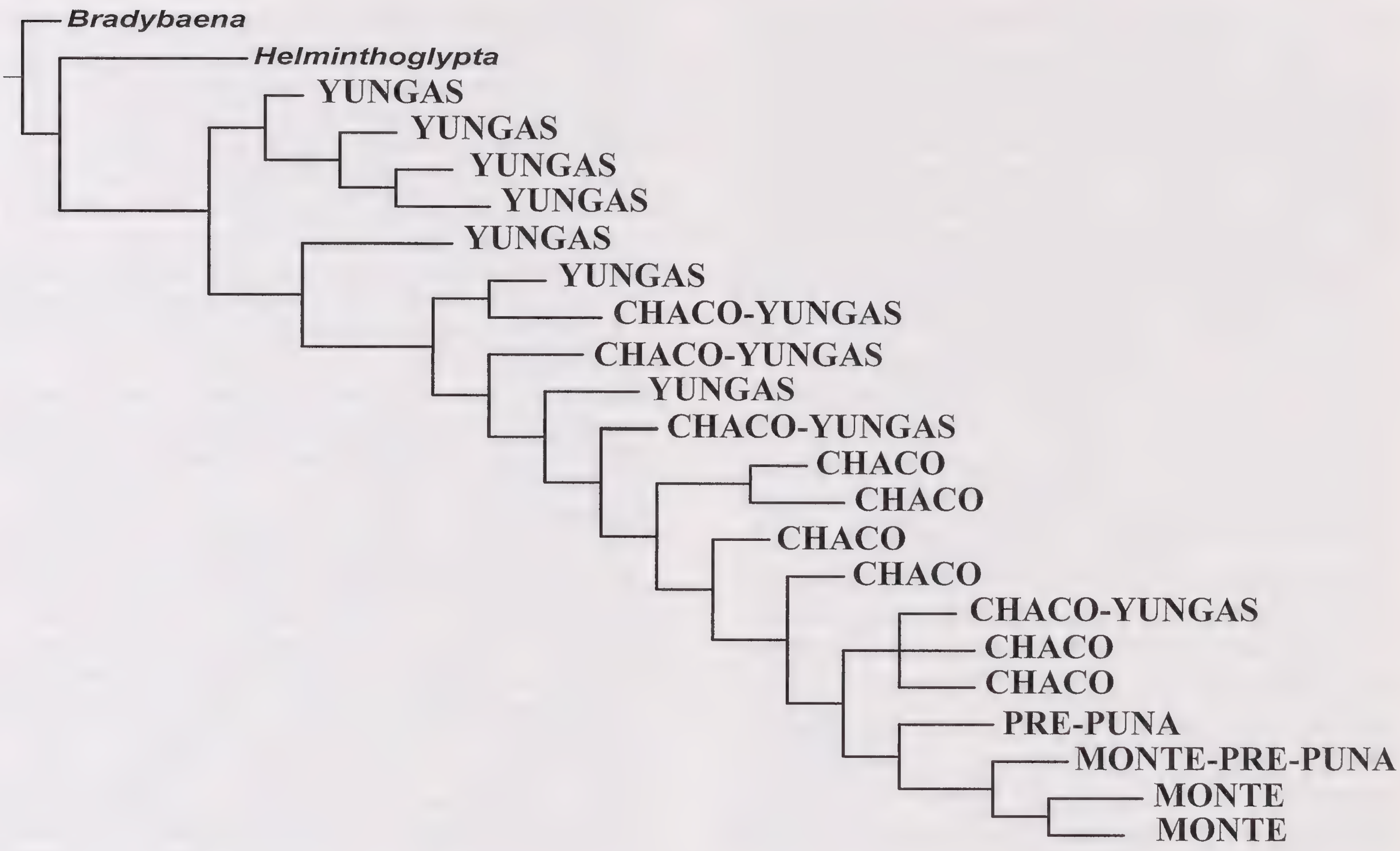


FIG. 24. Area cladogram of the *Epiphragmophora* species studied based on the preferred cladistic hypothesis obtained (Fig. 23).

area is very low, it has been postulated that this area presents a high degree of endemicity (Roig Juñet et al., 2002). *Epiphragmophora villavilensis* and *E. birabeni* are endemic species of the Pre-Puna in Argentina. *Epiphragmophora villavilensis* and *E. hieronymi* are sister groups of *E. puella* and ***E. quirogai*** that are habitants of the Monte province. *Epiphragmophora hieronymi*, usually an inhabitant of the Monte province, is also found in Pre-Puna locations of Catamarca Province. The relationships between Boreal Monte, the Chacoan areas of endemism and the Pre-Puna province remain unresolved in the present cladogram, although they are located in a derived position respect to the Yungas areas. In conclusion:

(1) *Epiphragmophora* is a monophyletic genus, the monophyly of which is supported in the present hypothesis (Fig. 23) by six unambiguous synapomorphies. There are two main monophyletic subclades within the genus that could potentially represent subgenera.

(2) Anatomical characters specially concerning the distal portion of the reproductive tract are more informative than shell characters to establish phylogenetic relationships. A new cladistic analysis is recommended only when the anatomy of the rest of the species presently known by shell descriptions or by single specimens is available.

(3) The insertion of the dart sac is either in the atrium or in the vagina and the two unequal mucous glands insert in the medial zone of a single dart sac.

(4) According to the area cladogram (Fig. 24) based on the phylogenetic hypothesis obtained, the rainforest areas or Yungas biogeographic province are paraphyletic. The xerophilic areas of Monte, Chacoan and Pre-Puna biogeographic provinces are located in a more derived position respect to the Yungas in the present hypothesis. The Monte and Pre-Puna conforms a monophyletic clade.

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APPENDIX

Matrix of 29 taxa per 35 characters used in the cladistic analysis. Underlined taxa are those for which anatomical data are lacking and which are not considered in the second parsimony analysis.

	Character																																		
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Bradybaena	0	3	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0	1	1	0	0	2
Helminthoglypta	[04]	[03]	1	0	0	0	1	0	1	2	1	2	2	0	0	1	0	0	0	0	2	0	0	2	1	0	2	3	0	0	1	0	0	0	1
E. argentina	2	[01]	0	1	0	0	1	0	1	1	1	1	0	1	1	0	1	1	0	0	1	0	1	2	0	1	1	3	1	0	1	1	0	0	1
E. birabeni	3	2	1	1	1	0	0	0	1	1	?	?	?	?	1	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	1	?	?	?	?
E. costellata	3	3	0	1	2	0	1	2	1	2	?	?	?	?	1	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	0	?	?	?	?
E. cryptomphala	2	[01]	[12]	1	0	0	2	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	2	0	0	0	0	1	0	1
E. escoipensis	0	1	1	0	1	0	[01]	0	1	1	1	0	1	1	1	1	1	0	1	1	0	0	1	1	0	0	0	2	0	0	1	0	0	0	1
E. hemiclausa	2	1	0	1	0	0	1	0	1	1	1	1	0	1	1	0	1	0	0	0	0	1	1	2	0	1	0	2	1	0	1	1	0	0	1
E. guevarai	0	2	0	0	1	0	2	0	1	1	1	0	1	1	1	1	1	0	0	1	2	1	1	0	0	0	0	2	0	0	0	0	1	0	1
E. hieronymi	0	2	0	0	1	0	1	0	0	1	1	0	1	1	1	1	1	0	1	1	1	1	2	0	0	0	1	2	1	0	0	0	0	1	1
E. jujuyensis	2	1	1	1	0	1	1	0	1	2	1	0	1	1	1	0	1	1	0	0	2	1	1	0	0	0	1	2	0	0	1	1	1	1	1
E. parodizi	3	1	0	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	2	0	0	1	0	0	0	2	0	0	1	0	1	0	1
E. proseni	2	1	1	1	1	0	0	0	1	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?
E. puella	4	2	0	2	1	0	0	1	0	0	1	0	?	?	1	?	?	0	1	?	?	?	?	0	0	?	?	2	?	?	0	0	1	?	?
E. puntana	3	1	1	0	0	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	2	1	1	1	0	0	0	2	0	0	0	0	0	0	1
E. quirogai	0	2	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	0	1	1	0	1	2	0	0	0	1	2	0	0	0	1	1	1	1
E. rhathymos	2	3	0	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1	0	0	0	2	0	0	0	0	1	0	1
E. saltana	3	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	0	1	0	1	1	0	0	0	2	0	1	1	0	0	0	1
E. tomsici	2	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	3	0	1	0	0	1	0	1
E. trenquelleonis	0	1	0	0	1	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	2	1	1	0	0	2	1	2	0	0	[01]	0	1	0	1
E. trifasciata	1	2	0	0	1	0	2	0	1	1	1	0	1	1	1	1	1	0	0	1	2	1	1	0	0	0	1	0	0	0	0	0	1	0	1
E. trigrammephora	[02]	2	2	0	0	0	2	0	1	1	1	0	1	1	1	1	1	0	0	1	2	1	1	0	0	0	1	2	0	0	[01]	0	1	0	1
E. tucumanensis	2	1	[01]	1	0	[01]	1	0	1	2	1	1	0	1	1	0	1	1	0	0	0	0	1	1	0	1	0	3	1	1	1	1	0	0	1
E. variegata	2	[01]	0	1	0	0	[012]	0	1	1	1	1	0	1	1	0	1	1	0	0	2	0	1	2	0	1	0	2	0	0	1	1	0	0	1
E. villavilensis	0	2	2	0	2	0	0	2	0	1	1	0	1	1	1	1	1	0	1	1	2	1	1	0	0	0	1	2	?	0	0	?	1	0	1
E. walshi	2	3	0	0	1	0	2	0	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	2	0	0	0	0	1	0	1
E. audouini	2	1	1	1	0	0	0	[01]	1	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?
E. estella	2	1	1	0	0	0	1	0	1	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
E. oresigena	2	1	2	1	0	0	2	0	1	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

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FRESHWATER GASTROPODA FROM ARGENTINA: SPECIES RICHNESS, DISTRIBUTION PATTERNS, AND AN EVALUATION OF ENDANGERED SPECIES

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ABSTRACT

Few studies have dealt with the geographic distribution of freshwater snails in Argentina. The objectives of this study were to: (1) determine species and family richness and diversity patterns; (2) verify if species richness behaves according to Rapoport's rule; (3) identify and classify species according to their distributions; and (4) identify endangered species. A grid was applied to a map of Argentina, with each of 340 squares (SUs) representing 10,000 km^2. A database of 3,376 records was analyzed. Of the 101 species belonging to ten families recorded in Argentina, four are introduced and 40 are endemic to Argentina. The Lithoglyphidae have the highest number of species (22). The highest species richness/SU was recorded in the Brazilic subregion at Salto Grande (32 species) on Uruguay River, and parts of the Río de la Plata (31). In this subregion the species richness values increase from west to east. The highest richness in the Chilean-Patagonian subregion was detected at San Carlos de Bariloche, Río Negro province (9), and the Chilinidae contained the largest number of species. In Argentina, the north-south decline species pattern could be explained through Rapoport's rule. In Patagonia, the species richness gradients do not show significant west-east trends. Most of the native species of freshwater molluscs of restricted distribution can be considered endangered (about 45 species) but need further study.

Key words: freshwater Gastropoda, native species, richness, distribution, Del Plata basin, Patagonia, Argentina.

INTRODUCTION

Natural ecosystems are progressively being destroyed, leading to a loss of biodiversity. A total of 1.4 million living species have been recorded (Wilson, 1988), of which, about 135,000 are molluscs, and of these more than 2,000 are known to be at risk of extinction (Seddon, 1998). The Molluscs Specialists Group (MSG) of the World Conservation Union (International Union for Conservation of Nature - IUCN) provides advice and data to the Species Survival Commission (SSC) of the IUCN, and through it, to the IUCN "Conservation Program for Sustainable Development". The aim of this program is to ensure the conservation of natural resources within the concept of sustainable development (Kay, 1995).

Argentina occupies most of the southern portion of South America, with an area of 2,780,403 km^2 and a population of 36 million people (National Census, 2001). The climate varies from sub-tropical in the north to cold-temperate in the south. Three of the ecoregions (biomes) with the greatest biodiversity in South America have their southern distributional limit in Argentina: the Paranaense and Yungas rainforests and the wetlands of Chaco (Brown & Grau, 1993; Bucher & Chain, 1998; Bertonatti & Corcuera, 2000). Molluscs were not included among the taxa considered by these authors in making these biodiversity assessments. These three ecoregions include the main rivers of the Del Plata basin. The distribution and quality of the different types of continental waters reflect the

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major hydrographic subregions to which they belong and are important in determining the distribution and composition of the freshwater mollusc faunas.

While the overall taxonomic composition of the freshwater gastropod fauna of Argentina is fairly well known, little information is available on their geographic distributions of the species (Bonetto et al., 1990; Castellanos & Miquel, 1991; Rumi et al., 1997) and patterns of diversity. According to "Rapoport's rule" (Rapoport, 1982), species richness peaks near the Equator and declines toward the poles, suggesting that species richness should be greater in northern than in southern Argentina.

This paper is part of a series of studies with the objective of identifying for Argentina the native species of freshwater gastropods that are endangered or at risk of extinction and to define the areas or ecosystems that should be protected in order to preserve them. The purpose of this paper is to identify the major groups of native species on the basis of their richness and distribution. With a macroecological focus, four objectives were defined in order to: (1) determine patterns of species and family richness and diversity; (2) verify if species richness behaves according to "Rapoport's rule"; (3) identify and classify groups of species according to their distributions; and (4) identify endangered species.

METHODS

Rivers in South America are distributed in two subregions: Brazilic in the north and Chilean-Patagonian in the south (Bonetto, 1994). There is a Transitional Zone between them, which has no characteristics of its own and includes primarily the Salí-Dulce, Primero and Segundo rivers of Córdoba Province, Argentina, which flow into the Mar Chiquita pond and form the greatest endorheic basin in South America. In Argentina (Fig. 1), the Brazilic subregion includes the Del Plata basin, and the Chilean-Patagonian subregion includes various lakes and rivers that mainly flow into the Atlantic Ocean.

A Gauss-Krüger map projection that divides the world into bands was used. A grid was applied to the map, with each square a sampling unit (SU) representing 10,000 km^2 (100 x 100 km) (Rabinovich & Rapoport, 1975; Heller & Kadmon, 2004). Argentina has 340 SUs, of which 19 (5.6% of the total area of the country) fall in dry zones without any riverbeds or permanent freshwater habitats and which were therefore excluded from the analysis.

Molluscan records were taken from several sources, including: (1) collections in the Museo de La Plata, Buenos Aires Province, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires city, and "Miguel Lillo" Foundation, Tucumán Province; (2) information obtained from sampling field trips; and (3) data reported in the literature, including large expedition reports and revisionary works. A "record" is a species collected at one place each time. Appendix 1 shows the collection material revised and the taxonomic criteria employed. Diversity of species of freshwater Gastropoda, ignoring subspecific distinctions, was expressed as the number of species per family per SU.

The number of species and families per SU were considered as units for richness (*S*) analysis. To show richness grid-maps, data were gathered at intervals of five species. Both north-south and west-east gradient analyses were performed, excluding SUs without any records. The average and range of species richness was estimated for each row and column of the grid, thereby removing the effect of differing area in each row/column. For both trend lines obtained the coefficient of determination was calculated (R^2 is a proportion of the total variation in Y that is explained or accounted for by the fitted regression). The distribution of species among families in each SU, is a measure of taxonomic diversity, which we estimated by means of the Shannon diversity index ($H = -\Sigma p_i \ln p_i$). Thus, for each SU, we did not evaluate specific diversity in the usual manner, in which each species is weighed by the number of individuals, but rather, we considered the distribution of species in families (Rabinovich & Rapoport, 1975), so that p_i = (number of species of family *i*) / (total number of species of SU). Thus, a zero value of *H* represents occurrence of only one family in the SU.

The following terms were used to characterize each species' distribution: endemic – species reported only from Argentina; restricted - species only present in 1 to 3 SUs; continuous – species that occur in adjacent SUs or in SUs that belong to the same river basin; discontinuous – species that occupy isolated SUs or in different river basin; exotic – species that are not native to Argentina.

Following UICN (1994), the list of endangered native species was developed as those species with a limited distribution in particular areas and those that had not been recorded since 1975.

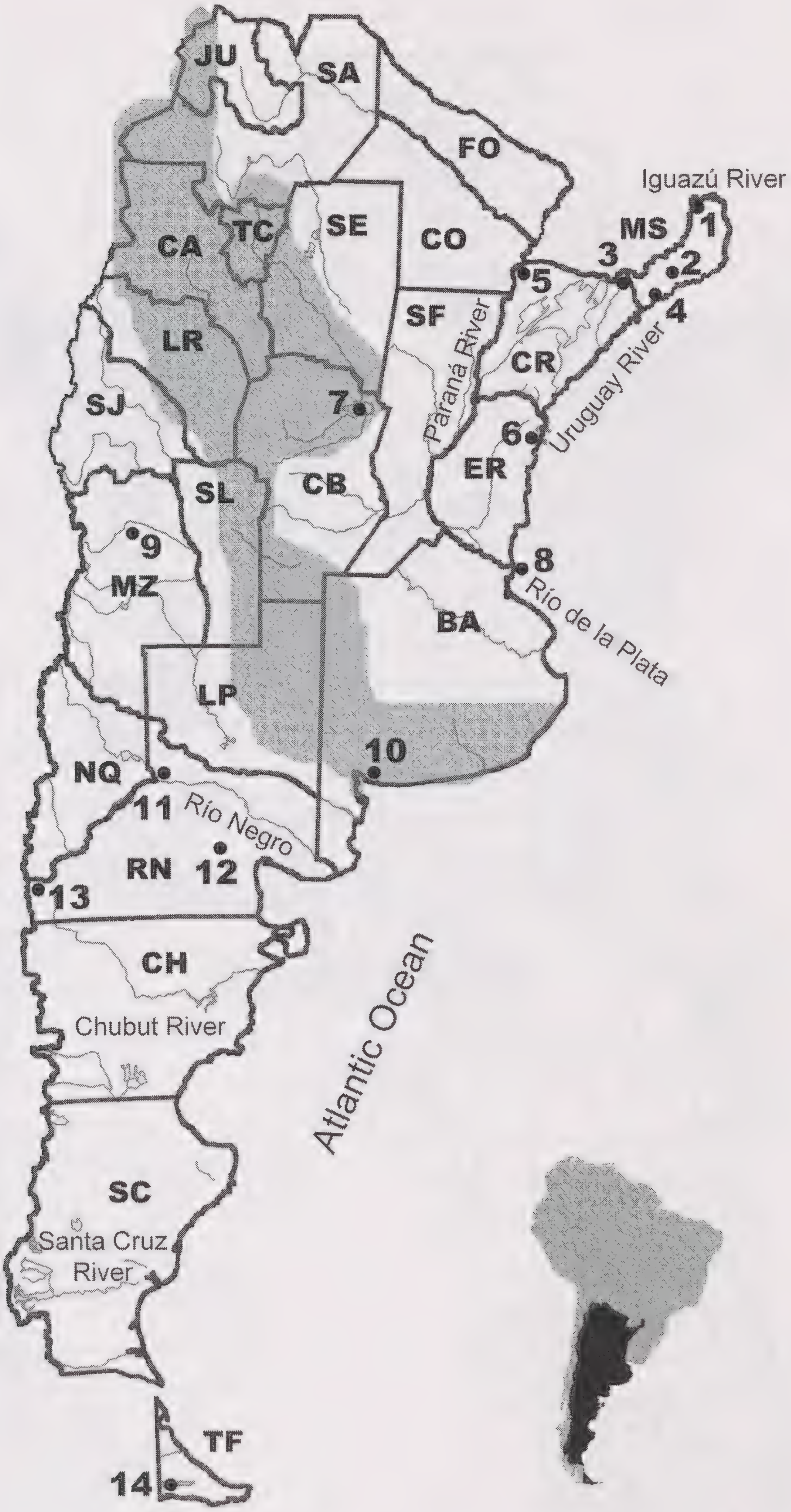

FIG. 1. Distribution of the rivers of Argentina. In grey, the Transitional Zone; to the north and east of it, the Brazilic subregion with the Del Plata basin; to the south and west of it, the Chilean – Patagonian subregion (adapted from Bonetto, 1994). Localities indicated as follows: 1 – Parque Nacional Iguazú; 2 – Salto Encantado; 3 – Yacyretá – Posadas; 4 – San Javier; 5 – Resistencia; 6 – Salto Grande; 7 – Mar Chiquita pond; 8 – Martín García Island; 9 – Mendoza city; 10 – Bahía Blanca; 11 – Neuquén-Cipolletti; 12 – Valcheta; 13 – San Carlos de Bariloche and Gutiérrez Lake; 14 – Yehuín Lake. BA: Buenos Aires; CA: Catamarca; CB: Córdoba; CH: Chubut; CO: Chaco; CR: Corrientes; ER: Entre Ríos; FO: Formosa; JU: Jujuy; LP: La Pampa; LR: La Rioja; MS: Misiones; MZ: Mendoza; NQ: Neuquén; RN: Río Negro; SA: Salta; SC: Santa Cruz; SE: Santiago del Estero; SF: Santa Fe; SJ: San Juan; SL: San Luis; TC: Tucumán; and TF: Tierra del Fuego.

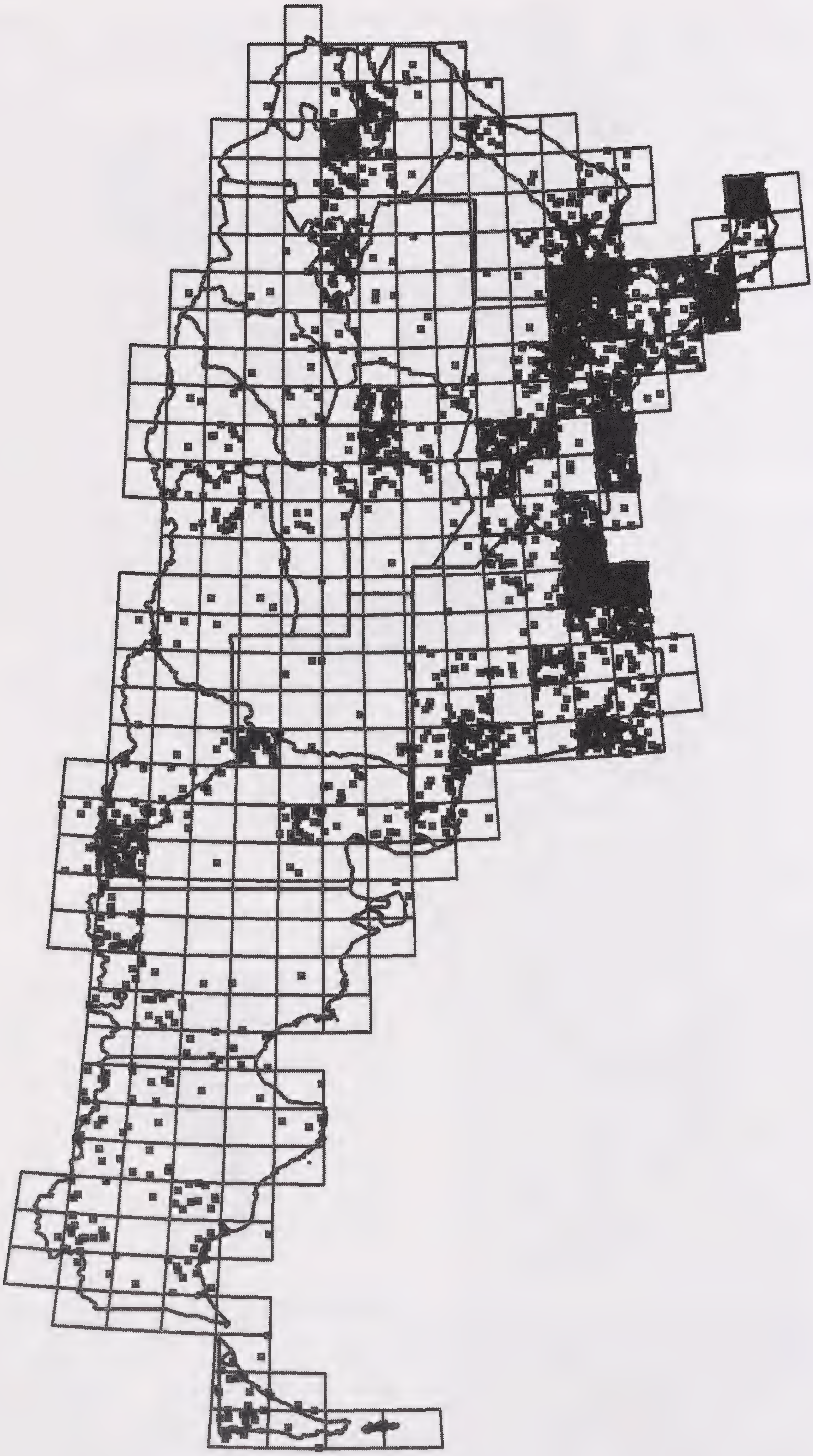

FIG. 2. Record density in each SU.

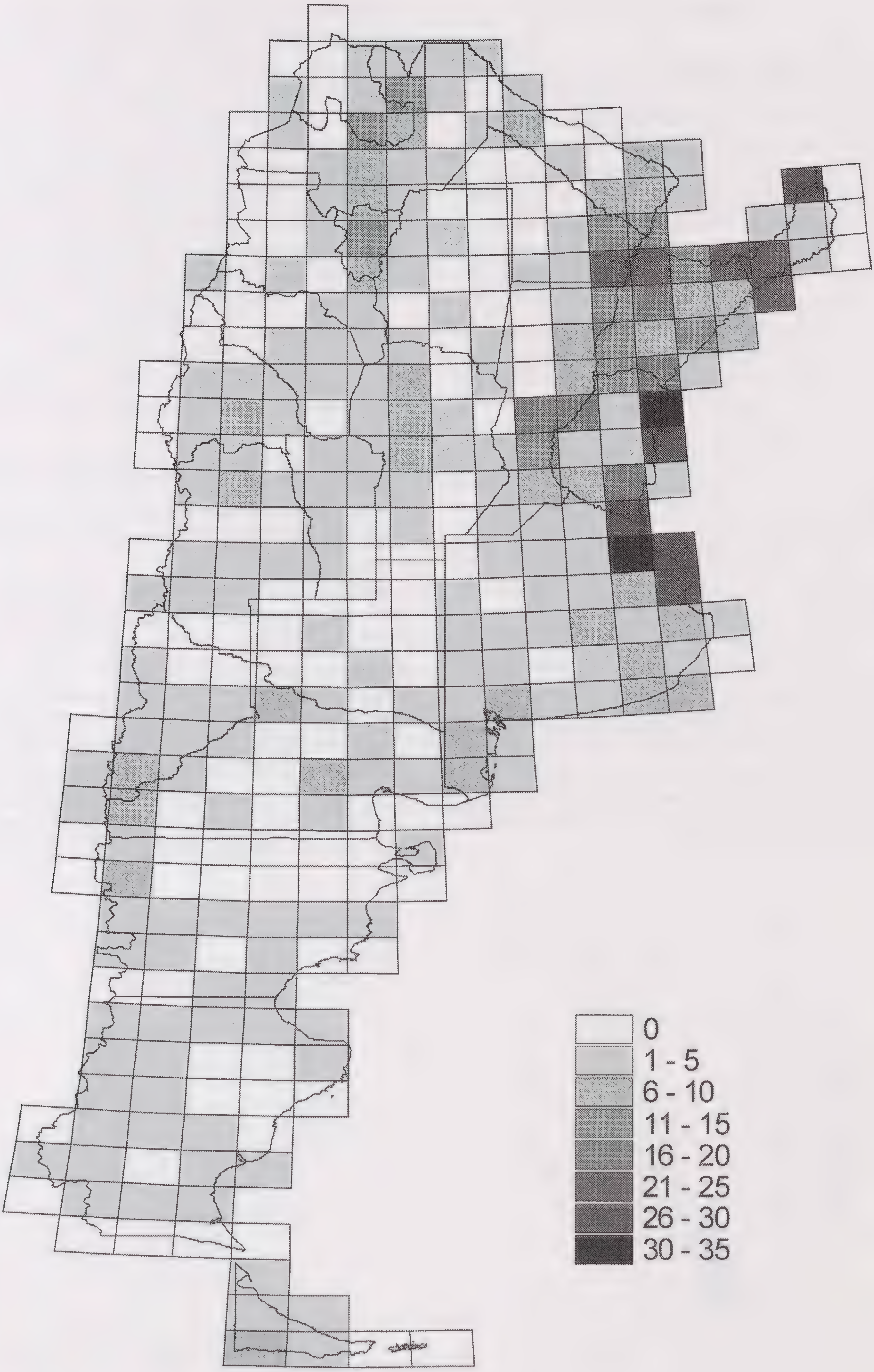

FIG. 3. Species richness in each SU.

RESULTS

The database includes a total of 3,376 records. Fig. 2 shows the number of records per SU, with randomly distributed spots within each SU. The largest number of records is from the Río de la Plata (219) and Parque Nacional Iguazú (216). Of the total of 340 SUs, 102 have no records (either because no freshwater species were found, or because the SU was not sampled); 26 are from the Brazilic subregion, 23 are from the Transitional Zone, and 53 are from the Chilean-Patagonian subregion.

A total of 101 nominal species belonging to ten freshwater gastropod families have been reported from Argentina: Ampullariidae (12 species), Cochliopidae (16), Lithoglyphidae (22), and Thiaridae (4) (Caenogastropoda); Ancylidae (5), Chilinidae (16), Glacidorbidae (1), Lymnaeidae (5), Physidae (5), and Planorbidae (15) (Heterobranchia). The Brazilic subregion has 75 species, the Chilean-Patagonian subregion 36, and the Transitional Zone 26. Of these species, 13 are found in all three regions. An additional 11 are found in two regions, three of them in the Transitional Zone and the Chilean-Patagonian subregion, seven in the Transitional Zone and the Brazilic subregion, and one in the Chilean-Patagonian and Brazilic subregions. Some species were found only in the Brazilic subregion (54), the Chilean Patagonian subregion (16), or the Transitional Zone (3).

The highest species richness per SU was found at Salto Grande (32 species), parts of the Río de la Plata (31), Parque Nacional Iguazú (26), Yacyretá-Posadas (25), San Javier, and at the confluence of the Paraná and Paraguay rivers (22), all of them belonging to the Brazilic subregion (Fig. 3). The Lithoglyphidae had the highest number of species in this subregion (21). In the Chilean-Patagonian subregion, the highest species richness was at San Carlos de Bariloche, Río Negro Province (9), and the Chilinidae had the highest number of species (10). In the Transitional Zone, the highest richness was in the centre of Tucumán Province (11) and the Cochliopidae had the highest number of species (9).

The correlation between record densities and species richness per SU (r = 0.87; N = 218) as seen in Figure 4. The highest richness value (32) corresponds to 122 records (3.8 times the richness value).

Family richness reached a maximum in the Yacyretá-Posadas area (9 families), Río de la Plata (8), Salto Grande (8), and Parque Nacional Iguazú (8), all in the Brazilic subregion. In the Chilean-Patagonian subregion the greatest family richness (6) was found at San Carlos de Bariloche, Mendoza city, and at the confluence of the Neuquén, Limay and Negro rivers at the cities of Neuquén and Cipolletti. In the Transitional Zone, the highest richness (7) was recorded from Bahía Blanca (Fig. 5).

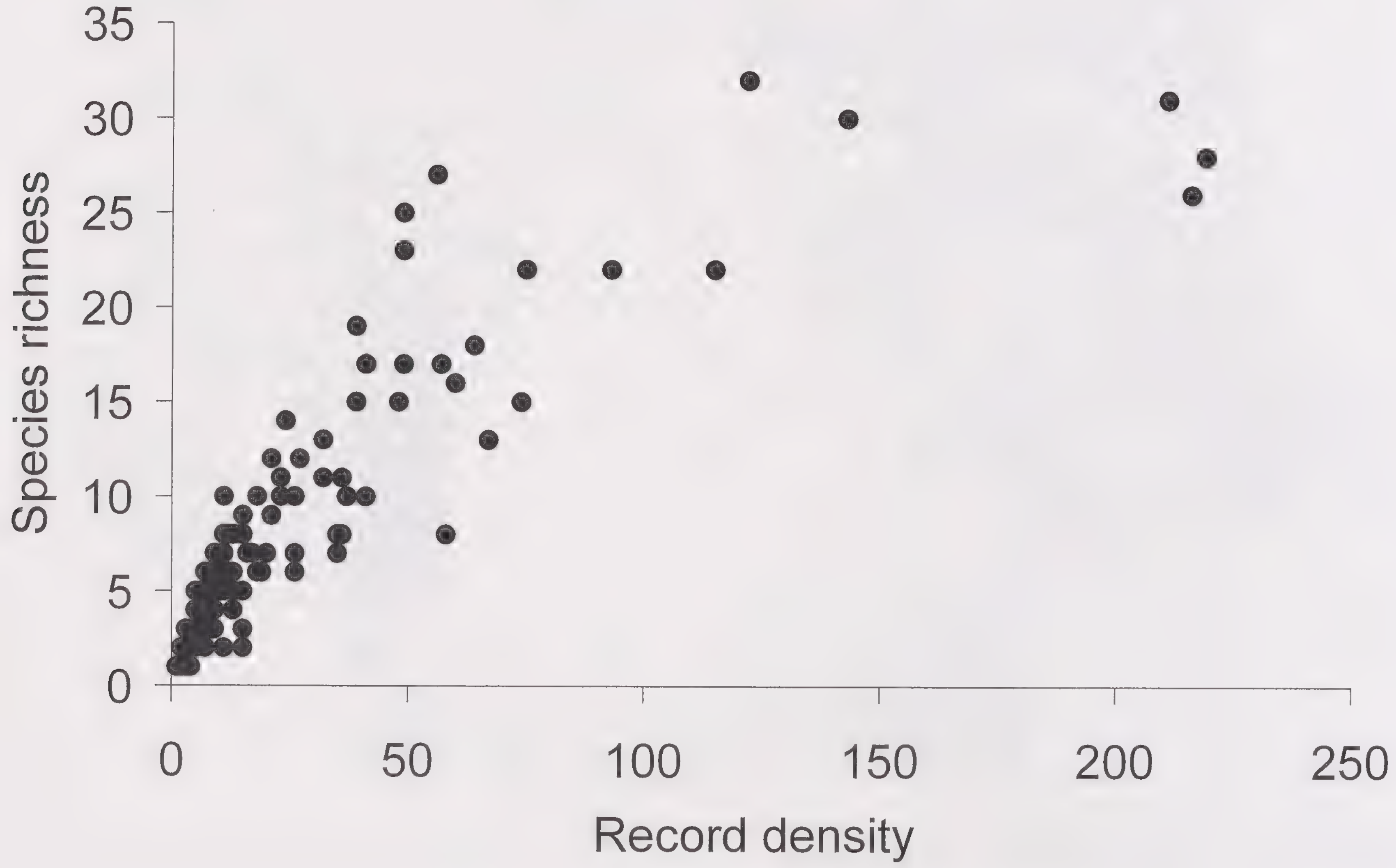

FIG. 4. Correlation between record densities and species richness.

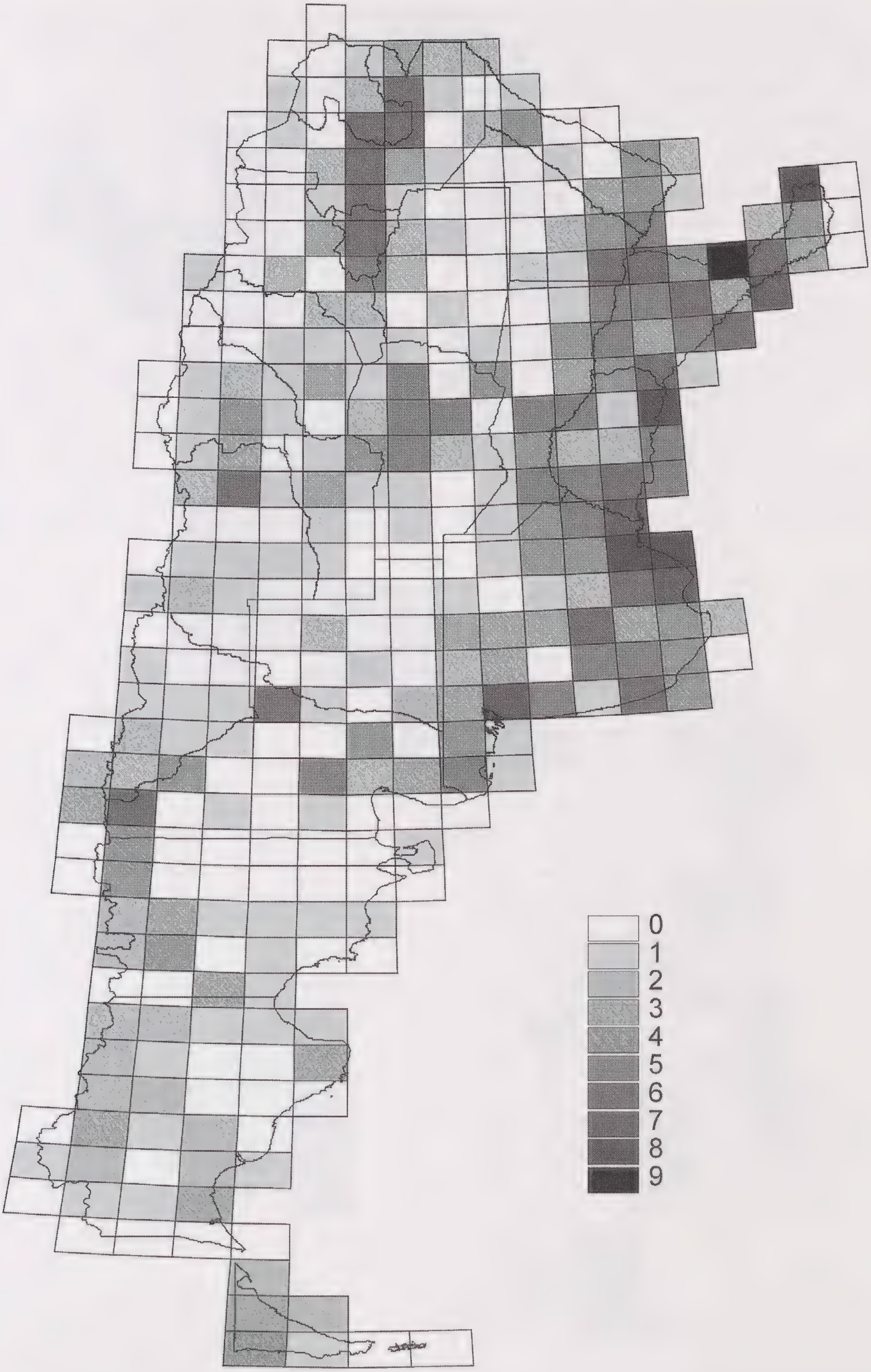

FIG. 5. Family richness in each SU.

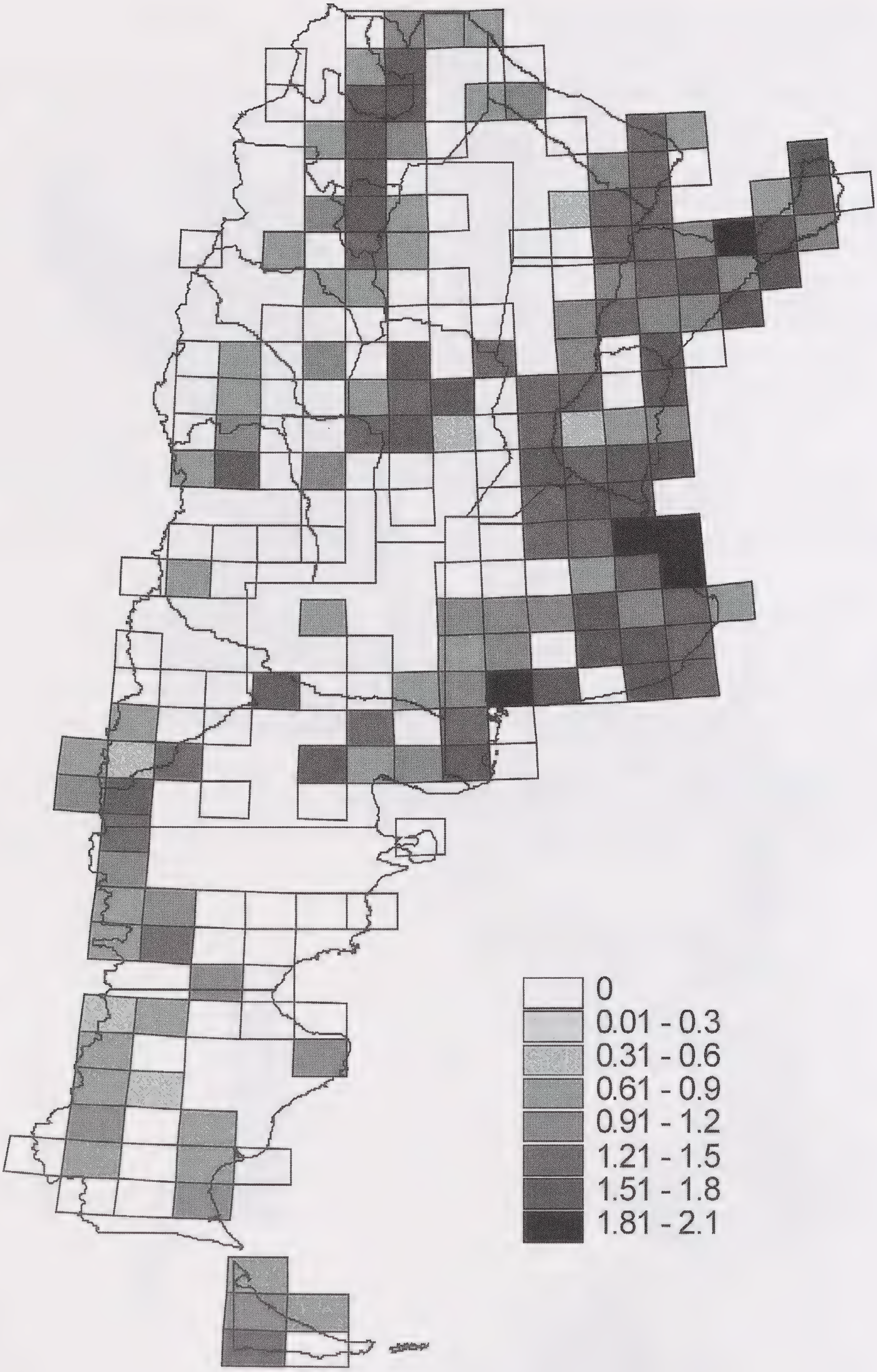

FIG. 6. Shannon diversity index value for each SU. Any SU own values of diversity in the interval 0.01–0.3.

The highest Shannon diversity indices were found in Yacyretá-Posadas ($H = 2.05$) and Río de la Plata ($H = 1.91$) (both in the Brazilic subregion) (Fig. 6). In the Chilean Patagonian subregion, the highest diversity indices were found at Mendoza city ($H = 1.79$) and Neuquén and Cipolletti cities ($H = 1.67$). In the Transitional Zone, the highest diversity index was in Bahía Blanca ($H = 1.83$), with a relatively low species richness (10 species). Seventy-five SUs had a diversity index of zero.

The north-south gradient of maximum and means of species richness is shown in Fig. 7A. Both regression of species richness per grid row on latitude exhibited a negative slope (b), with a low coefficient of determination ($R^2 = 0.37$; $P << 0.005$). The b absolute value for maximum richness ($b = -0.53$) is higher than b obtained for means ($b = -0.13$). In the north, the low values of richness correspond to a desert area (rows = 1–4), while the highest mean values per row occur in areas of the Yungas rainforests in the north-west and Paraná river in the north-east between Resistencia and Posadas (mean $S = 10.17$; range = 1–25; row = 7). The ranges of species richness rise towards the south of Del Plata basin (rows = 11–15), from the centre, the mean per row of SUs diminishes (row = 16). The decline in mean and range of species richness becomes more evident towards Patagonia. The highest value of richness by row ($S = 7$; row = 24) in Patagonia is from a row with data in only one SU. The north-south distribution of family richness (Fig. 7B) exhibits a similar pattern to that of species richness (maximum: $R^2 = 0.51$, $b = -0.13$; mean: $R^2 = 0.32$, $b = -0.04$; $P << 0.005$).

The west-east gradient of species richness is shown in Fig. 8A: it increases from west to east (maximum and mean: $R^2 = 0.64$; $P << 0.005$; maximum: $b = 1.36$; mean: $b = 0.74$),

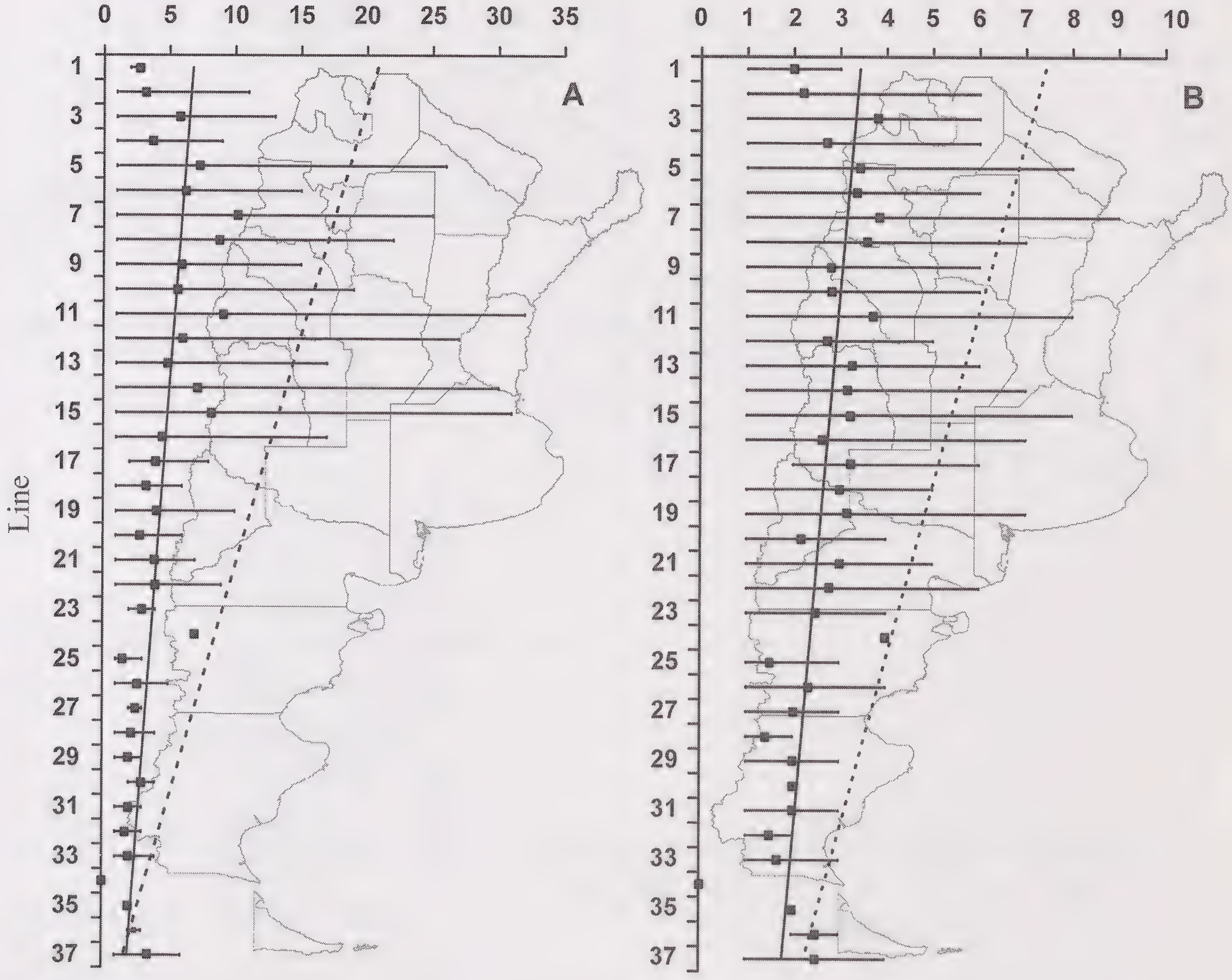

FIG. 7. North-south richness gradients expressed as mean (black dots and continued line), maximum (dashed), and range of values for SUs in each row of SUs. A: Species; B: Families.

with a greater overall range than in the north-south gradient. If only the northern region is considered (Fig. 8B), comprising mainly the big rivers of the Del Plata basin (Paraná, Uruguay and Río de la Plata), there is a strong increase in species richness from west to east (maximum: $R^2 = 0.73$; $b = 1.68$; mean: $R^2 = 0.72$; $b = 0.84$; $P << 0.005$). However, when only the southern area is considered (Fig. 8C) no trend was found (maximum: $R^2 = 0.28$; $b = -0.4$; $P = 0.12$; mean: $R^2 = 0.0007$; $b = 0.01$; $P = 0.95$). The ranges of richness are higher in the west, along column two where are located the majority of the great lakes, and along column five around the cities of Neuquén and Cipolletti and at some areas of Tierra del Fuego Province.

There are forty (40) endemic species in Argentina: Thiaridae (3), Ampullariidae (1), Cochliopidae (10), Lithoglyphidae (11), Chilinidae (11), Lymnaeidae (2), and Physidae (2). Notably, of the species of Lithoglyphidae, Cochliopidae, and Chilinidae, 50%, 62.5%, and 68.7%, respectively, are endemic. Among the 45 species evaluated as endangered on the basis of the criteria given in the Methods (Table 1), 32 are endemic, ten have only been reported from their type localities, 19 have no recent records, 25 have a continuous restricted distribution and six a discontinuous one. Fifteen of these endangered species occur in protected areas.

Only four exotic freshwater gastropod species are reported in Argentina: *Physella*

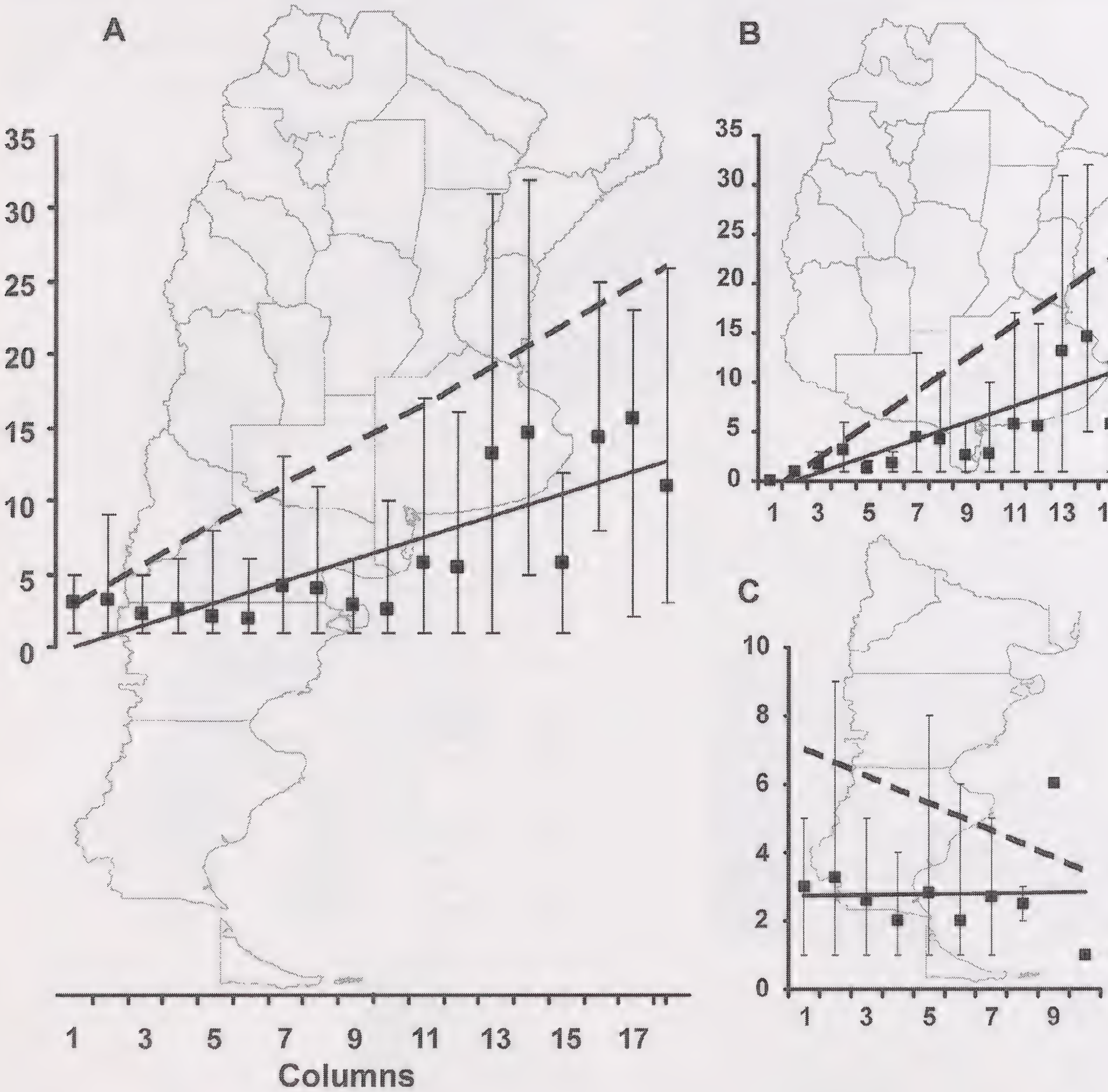

FIG. 8. West-east species richness gradient expressed as mean (black dots and continued line), maximum (dashed), and range of values for SUs in each row of SUs. A: All of Argentina; B: northern Argentina; C: southern Argentina.

cubensis (Pfeiffer, 1839), *P. venustula* (Gould, 1848) (Miquel & Zelaya, 1999) (Physidae); *Lymnaea columella* (Say, 1817) (Lymnaeidae); and *Melanoides tuberculata* Müller, 1758 (Thiaridae) (Castellanos & Miquel, 1991; Quintana et al., 2001–2002).

TABLE 1. Endangered native species: CRD: continuous restricted distribution; DRD: discontinuous restricted distribution; E: endemic; PA: species occurring in protected areas; TL: only known from the type locality; WRR: without recent records. Species authors and dates are included in Appendix 1.

Family	Species	E	TL	WRR	CRD	DRD	PA
Thiaridae	*Aylacostoma chloroticum*	X	X				
	A. guaraniticum	X	X				
	A. stigmaticum	X	X				
Ampullariidae	*Asolene platae*	X			X		
	Felipponea elongata				X		
	F. iheringi				X		
Cochliopidae	*Heleobia castellanosae*	X		X	X		
	H. conexa	X				X	
	H. isabelleana			X	X		
	H. kuesteri	X			X		
	H. montana	X		X	X		
	H. occidentalis	X		X		X	
	H. peiranoi	X	X	X			
	H. rionegrina	X	X	X			X
	H. tucumana	X		X			
	H. sublineata	X	X	X			
	H. vianai	X		X			
Lithoglyphidae	*Potamolithus concordianus*	X	X	X			
	P. valchetensis	X	X				X
	P. quadratus			X	X		
	P. tricostatus	X		X	X		X
	P. agapetus	X			X		
	P. dinochilus	X			X		
	P. catherinae				X		
	P. conicus	X			X		X
	P. doeringy				X		
	P. hidalgoi	X			X		
	P. microtauma				X		
	P. orbignyi				X		X
	P. bushii	X			X		X
	P. simplex	X			X		X
Glacidorbidae	*Gondwanorbis magallanicus*					X	X
Chilinidae	*Chilina aurantia*			X	X		X
	C. guaraniana	X	X	X			
	C. megastoma	X				X	X
	C. neuquenensis	X			X		X
	C. perrieri	X		X	X		
	C. portillensis	X		X	X		
	C. strebeli	X				X	X
Lymnaeidae	*Lymnaea plicata*	X	X	X			
Planorbidae	*Acrorbis petricola*					X	X
Ancylidae	*Gundlachia ticaga*				X		X
	Laevapex sp.				X		X
Physidae	*Physa aspii*	X		X			
	P. loosi	X		X			

DISCUSSION

The areas of greatest diversity of freshwater gastropods do not correspond exactly with the three ecoregions cited as having the greatest diversity in Argentina (Paranaense and Yungas rainforest and Chaco wetlands) (Brown & Grau, 1993; Bucher & Chain, 1998; Bertonatti & Corcuera, 2000). For gastropods, the SUs with the greatest diversity are found in the ecoregion of Delta and Paraná islands (Yacyretá - Posadas and Río de La Plata SUs) (Brazilic subregion) and in La Pampa ecoregion (Bahía Blanca) (Transitional Zone). Paranaense and Yungas rainforest follow in importance in diversity values. With respect to the Paranaense rainforest, Bonetto & Hurtado (1999) reported two species of *Aylacostoma* (Thiaridae) and many species of Chilinidae and *Heleobia* from the Iguazú River and its falls. However, the present work does not confirm the presence of *Aylacostoma* species in the Iguazú River. Also, in this study only one species of Chilinidae (*Chilina megastoma* Hylton Scott, 1958) and one undetermined species of *Heleobia* were recorded from the Iguazú River.

The Del Plata basin has the highest richness and diversity in Argentina, because three groups of gastropods with clearly different distribution patterns co-occur in this region. The first group includes species of Planorbidae, Ampullariidae, Thiaridae, Physidae, and Ancylidae, families with wide distribution that reach their southern limit in this area. Among the Planorbidae, *Drepanotrema* spp. is an endemic group from southern Texas to South America (Baker, 1945), and *Biomphalaria* spp. are also found in Africa and North America. The Ampullariidae are found in Africa, Asia, and Central and South America. The Thiariidae occur in South America, Africa, and Asia. The Physidae and Ancylidae are the most cosmopolitan groups. The second group comprises the Chilinidae, which has a southern distribution, with its greatest richness in the Chilean-Patagonian subregion, but which reaches its northern limit in northeast Argentina around the Tropic of Capricorn. The Lithoglyphidae constitute the third group and are almost entirely endemic to this basin, being most prevalent in the Uruguay River.

The absence of species of the second and third group beyond the Tropic of Capricorn, and the presence in the south of species of the first group, several coming from Holartic region, support the suggestions of Parodiz (1977) that at the beginning of the Tertiary, a very rich fauna of freshwater molluscs and mammals were involved in a unique migratory in a north-south orientation.

According to Bonetto (1994), the Transitional Zone, which has no characteristics of its own, only includes the Central Endorheic basins. Its fauna is more similar to the Brazilic one, as has been observed for fishes (A. Scione, pers. comm.). This zone marks the western distributional limit of the Paranoplatensean (the Del Plata basin) ichthyofauna, with species of Characidae and Loricariidae (López et al., 2002). The Ampullariidae have a similar distributional limit. The Transitional Zone shares seven species of freshwater gastropods with the Brazilic subregion but only three with the Chilean-Patagonian subregion.

In the Chilean-Patagonian subregion, besides the Chilinidae, there is one species of Glacidorbidae, *Gondwanorbis magallanicus* (Meier-Brook & Smith, 1976). This family, with about 20 species in Oceania (Ponder & Avern, 2000), constitutes a disjunctive element of distribution, that is, South America-Oceania.

In southern South America a north-south latitudinal gradient in species richness is a widely recognised pattern for many taxa and habitats (Taylor & Gaines, 1999). Brazil extends from 5°N to 34°S and harbours about 181 native species and subspecies and six exotic species of freshwater snails (M.C. Mansur, per. comm.). To the south, Argentina, extending from 22°S to 55°S, has 97 native species and four exotic taxa. This decrease between Brazil and Argentina and within Argentina could be explained through Rapoport's rule. The increase of species richness from west to east is evident only in the northern part of Argentina, where the eastern area includes the big rivers of the Del Plata basin. When analysing the southern part of Patagonia, the highest richness values are found in the west, where the great lakes of the Andes act as reservoirs of this mollusc fauna, in contrast to the northern area, which lacks great lakes, but the south of Argentina is the shape of an inverted cone, wherein the Andean region and its lakes move to the southeast on the grid, thereby reducing the west-east richness gradient.

Regarding endemism in the freshwater gastropods of Argentina, Castellanos & Miquel (1991) considered there to be few endemic Basommatophora. Given that in Perú only nine species of freshwater Basommatophora are endemic (28.1%) (Ramirez et al., 2003), we consider that 15 species of Basommatophora endemic to Argentina (32.6%) is a significant number. Overall, 39.6% of the freshwater gastropod species of Argentina are endemic, with

9.9% belonging to the Cochliopidae, 10.9% to the Lithoglyphidae, and 10.9% to the Chilinidae.

Among the endangered species known only from their type localities, only the species of *Aylacostoma* (Thiaridae) have multiple records, all of them prior to the completion of the Yacyretá hydroelectric dam in 1990. This dam has greatly modified their natural habitat, turning a river with rapids and numerous falls into a lake. According to Quintana & Mercado Laczkó (1997), these species can be considered extinct in their natural habitat. The same could have happened with *Chilina guaraniana* Castellanos & Miquel, 1980, and *Acrorbis sp.*, also collected before the filling of the reservoir (Rumi, 1986).

Other species collected only from their type localities are *Heleobia rionegrina* (Gaillard, 1974) and *Potamolithus valchetensis* Miquel, 1998, from Valcheta stream of Somuncurá system (Chilean-Patagonian subregion). The latter species was found in the stomach of *Gymmocharacinus bergi* Steindachner, 1903, a fish endemic to the Somuncurá system. This snail is probably the only living species of *Potamolithus* that lives outside of the Del Plata basin. *Potamolithus australis* Biese, 1944 (type locality Llanquihue lake, Chile; Holotype: Museo Historia Natural de Chile 200,619), which according to López Armengol (1985), is a doubtful species as only one specimen without soft part (Holotype). However, within a historical context, *P. windhauseni* (Parodiz, 1961) was described for the Paleocene in Nahuel Niyeu, a locality that belongs to the Somuncurá system (Parodiz, 1969). The genus *Potamolithus* also includes fossil species from the Patagonia's early Tertiary, occurring at both sides of the Andes (Parodiz, 1965).

Most of the species lacking recent records occur in areas rarely sampled, with the exception of *H. castellanosae* (Gaillard, 1974), *H. isabelleana* (d'Orbigny, 1835), and three species of *Potamolithus* (*P. concordianus* Parodiz, 1966; *P. quadratus* Pilsbry & Ihering, 1911; *P. tricostatus* (Brot, 1967)). These have been recorded in areas where sampling was frequent, such as the Río de la Plata and Uruguay rivers. *Physa loosi* Holmberg, 1909, and *P. aspii* Holmberg, 1909, are represented each by only two records with incomplete geographic information.

In general, the endangered species discussed here need further morphological, systematic, biological, and ecological study in order to categorize them more adequately.

Two species with restricted distribution in Argentina but not considered as endangered as they are more widely distributed in South America are *Biomphalaria intermedia* (Paraense & Deslandes, 1962) and *B. oligoza* Paraense, 1974.

Among the exotic species, *Physella cubensis* (= *Physa acuta* Draparnaud, 1805) is a species introduced with the import and transportation of aquatic plants (Miquel & Zelaya, 1999). The rare findings of *P. venustula* in Argentina do not allow us to make a conclusion about its exotic or native character. *Melanoides tuberculata* is considered an invasive species in other neotropical areas (Fernández et al., 2003). This species was introduced to Texas, United States, in the 1960s (Murray, 1964), Brazil in the 1970s, and Argentina by the end of the 1990s, when it was found in the reservoir of the Yacyretá hydroelectric dam. Finally, we should mention the risk of introduction of *Helisoma duryi* (Wetherby, 1879) (Rumi et al., 2002) in Buenos Aires Province, because of the introduction and commercialization of aquatic plants with this snail species.

The relationship between record density and richness has an asymptotic tendency because the SUs with the highest numbers of records also had the highest richness, that is, the Rio de la Plata and Parque Nacional Iguazú SUs. These results can be interpreted that there is a low probability that more species in those areas could be found. The sparse records (SUs with no data) from southern Argentina may be due to few roads in the Southern-Patagonian and Desaguadero and Colorado rivers areas, making large areas inaccessible. At least for molluscs, as shown in Fig. 2, greater sampling effort (and biological studies) has been undertaken in the Río de la Plata than elsewhere in Argentina. Most of the records derive from exploratory expeditions at the beginning of the twentieth century. Further focused sampling programs are necessary. In spite of these problems, our results show general trends in freshwater gastropod richness and diversity that will help guide future actions related to their conservation.

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APPENDIX 1

Species list with taxonomic remarks. Material examined (record numbers) by collection: MLP (Museo de La Plata); MACN (Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"); FML (Fundación Miguel Lillo). *: Material from private collections; TM: Type Material; RM: Reference Material.

CAENOGASTROPODA Cox, 1960

Ampullariidae Gray, 1824	MLP	MACN	FML	Reference Lot
Gen. *Asolene* d'Orbigny, 1875				
A. (A.) platae (Maton, 1809)	15			MLP: 361 (RM)
A. (A.) pulchella (Anton, 1839)	19	34	1	MLP: 360 (RM)
A. (A.) spixii (d'Orbigny, 1835)	20		1	MLP: 10932 (RM)
Gen. *Felipponea* Dall, 1919				
F. elongata (Dall, 1921)	2			MLP: 6678 (RM)
F. iheringi (Pilsbry, 1933)	2	9		MLP: 8820 (RM)
F. neritiniformis (Dall, 1919)	8	3		MLP: 5338 (RM)
Gen. *Marisa* Gray, 1824				
M. planogyra Pilsbry, 1933	11	12		MLP: 359 (RM)
Gen. *Pomacea* Perry, 1810				
P. canaliculata (Lamark, 1822)	191	60	29	MLP: 281 (RM)
P. insularum (d'Orbigny, 1835)	2	7	1	MLP: 341 (RM)
P. scalaris (d'Orbigny, 1835)	31	23	4	MLP: 358 (RM)
Gen. *Pomella* Gray, 1847				
P. (P.) americanista (Ihering, 1919)	15	5	3	MACN: 8776 (TM)
P. (P.) megastoma (G. B. Sowerby I, 1825)	34	5	1	MLP: 1187 (RM)

Remarks: For the nomenclature of these species, the revision of Cowie & Thiengo (2003) was followed. *Pomacea insularum*: possible synonym of *Pomacea gigas* (Spix, 1827) (Castellanos & Fernández, 1976; Cowie & Thiengo, 2003). Lot MLP 11359 corresponds to the syntype of *P. canaliculata chaquensis*.

Thiaridae Troschell, 1857	MLP	MACN	FML	Reference Lot
Gen. *Aylacostoma* Spix, 1827				
A. chloroticum Hylton Scott, 1954	2			MLP: 10958 (TM)
A. guaraniticum (Hylton Scott, 1951)	3			MLP: 11213 (TM)
A. stigmaticum Hylton Scott, 1954	3			MLP: 10963 (TM)
Gen. *Melanoides* Oliver, 1804				
M. tuberculatus (Müller, 1774)	*			

Remarks: Castellanos (1981), with a synonymy of each species, and Quintana & Mercado Lackzó (1997) were followed. *Aylacostoma cingulatum* Hylton Scott (in schedule) was not taken into account as well as a new morphotype (Quintana & Mercado Lackzó, 1997), because there is as yet no proper description.

Cochliopidae Tryon, 1866	MLP	MACN	FML	Reference Lot
Gen. *Heleobia* Stimpson, 1865				
H. australis (d'Orbigny, 1835)	9	11		MLP: 4049 (TM)
H. castellanosae (Gaillard, 1974)	3			MLP: 4052 (TM)
H. conexa (Gaillard, 1974)	6			MLP: 4053 (TM)
H. guaranitica (Doering, 1884)	3			MLP: 4441 (RM)
H. hatcheri (Pilsbry, 1911)	18	3		MLP: 373 (RM)
H. isabelleana (d'Orbigny, 1835)		1		MACN: 28791 (RM)
H. kuesteri (Strobel, 1874)		4	2	FML: 5813 (RM)
H. montana (Doering, 1884)	1	1		MLP: 9153 (RM)
H. occidentalis (Doering, 1884)	1			MLP: 9097 (RM)
H. parchappii (d'Orbigny, 1835)	16	16		MLP: 11746 (RM)
H. peiranoi (Weyrauch, 1963)			1	FML: 41 (TM)
H. piscium (d'Orbigny, 1835)	9	14	1	MLP: 6760 (RM)
H. rionegrina (Gaillard, 1974)	1	1		MLP: 4050 (TM)
H. sublineata (Pilsbry, 1911)	*			
H. tucumana (Gaillard, 1974)	3		1	MLP: 4051 (TM)
H. vianai (Parodiz, 1960)	4	2		MLP: 387 (RM)

Remarks: According to Davis et al. (1982), *Littoridina australis* and the related species of Brazil, Uruguay and Argentina are included in the genus *Heleobia*. According to Kabat & Hershler (1993), *Strobeliella* Cazzaniga, 1981, is a synonym of *Heleobia*. The synonymic list of this family was published by Gaillard & Castellanos (1976) based on studies of the shell and penis morphology. Cazzaniga (1981) mentions *H. vianai* as a possible synonym of *H. occidentalis*. *Heleobia scotti* (Pilsbry, 1911) was not considered due to incomplete geographic information. *Helobia isabelleana* (type locality: Montevideo, Uruguay) was not included in the list of species of the Uruguay by Scarabino (2004).

Lithoglyphidae Troschel, 1857	MLP	MACN	FML	Reference Lot
Gen. *Potamolithus* Pilsbry, 1896				
P. agapetus Pilsbry, 1911	21	5		MLP: 4650 (RM)
P. buschii (Frauenfeld, 1865)	24	13		MLP: 4621 (RM)
P. callosus Pilsbry, 1925	3	1		MLP: 4615 (RM)
P. catharinae Pilsbry, 1911	*			
P. concordianus Parodiz, 1966		1		MACN: 17334 (RM)
P. conicus (Brot, 1867)	2	13		MLP: 4623 (RM)
P. dinochilus Pilsbry, 1896	1			MLP: 4615 (RM)
P. doeringi Pilsbry, 1911	6	2		MLP: 4617 (RM)
P. hidalgoi Pilsbry, 1896	2			MLP: 371 (RM)
P. iheringi Pilsbry, 1896	4	3		MLP: 4615 (RM)
P. lapidum (d'Orbigny, 1835)	19	26		MLP: 371 (RM)
P. microthauma Pilsbry, 1896	2	1		MLP: 9948 (RM)
P. orbignyi Pilsbry, 1896	1	4		MLP: 4623 (RM)
P. paranensis Pilsbry, 1911	6	3		MLP: 4614 (RM)
P. peristomatus (d'Orbigny, 1835)	7	1		MLP: 4618 (RM)
P. petitianus d'Orbigny, 1840	3	17		MLP: 4623 (RM)
P. philipianus Pilsbry, 1911	8	4		MLP: 4616 (RM)
P. quadratus Pilsbry & Ihering, 1911		1		MACN: 17338 (RM)
P. rushii Pilsbry, 1896	2	1		MLP: 4615 (RM)
P. simplex Pilsbry, 1911	2	1		MLP: 4623 (RM)
P. tricostatus (Brot, 1867)	2	1		MLP: 4615 (RM)
P. valchetensis Miquel, 1998		3		MACN: 3411 (TM)

Remarks: *Potamolithus jacuhyensis* Pilsbry, 1899, was mentioned by Parodiz (1965), but in later revisions it is considered present only in Brazil (López Armengol, 1985). Likewise, no lot was found in the collections consulted. López Armengol (1985) published the synonymy list of this family, which was then completed by López Armengol & Darrigran (1998).

HETEROBRANCHIA Burmeister, 1837

Glacidorbidae Ponder, 1986	MLP	MACN	FML	Reference Lot
Gen. *Gondwanorbis* Ponder, 1986				
G. magallanicus (Meier-Brook & Smith, 1976)	2			MLP: 5503 (RM)

Remarks: Only one species belongs to the Glacidorbidae in South America. According the revision of *Glacidorbis* by Ponder & Avern (2000), the genus is restricted to Australian species and the South American taxon is assigned to *Gondwanorbis*.

Chilinidae Gray, 1828	MLP	MACN	FML	Reference Lot
Gen. *Chilina* Gray, 1828				
C. aurantia Marshall, 1924		1		MACN: 23298 (RM)
C. dombeiana (Bruguière, 1789)	8	3		MLP: 769 (RM)
C. fluminea (Maton, 1809)	15	30		MLP: 386 (RM)
C. fulgurata Pilsbry, 1911	5	7		MLP: 2262 (RM)
C. gallardoi Castellanos & Gaillard, 1981	5	7		MLP: 4096 (TM)
C. gibbosa G. B. Sowerby I, 1839	16	25		MLP: 698 (RM)
C. guaraniana Castellanos & Miquel, 1980		1		MACN: 568 (TM)
C. megastoma Hylton Scott, 1958	9			MLP: 11068 (TM)
C. mendozana Strobel, 1874	3			MLP: 7391 (RM)
C. neuquenensis Marshall, 1933	1	1		MLP: 8262 (RM)
C. parchappii (d'Orbigny, 1835)	28	37		MLP: 764 (RM)
C. patagonica Sowerby II, 1874	4	2		MLP: 2903 (RM)
C. perrieri Mabille, 1833	2			MLP: 4102 (RM)
C. portillensis Hidalgo, 1880	2			MLP: 2967 (RM)
C. rushii Pilsbry, 1911	4	3		MLP: 8276 (RM)
C. strebeli Pilsbry, 1911	1	2		MLP: 6725 (RM)

Remarks: Castellanos & Gaillard (1981) prepared a synonymic list of this family. The material of *C. guaraniana* cited as deposited in the MLP collection by Castellanos & Miquel (1980) was not found in that collection. Lots 9927 (Paratype, Río Cochuna) (TC) and 9954 from MACN correspond to *C. fluminea tucumanensis*. At present, the species of this family are under revision.

Lymnaeidae Rafinesque, 1815	MLP	MACN	FML	Reference Lot
Gen. *Lymnaea* Lamarck, 1799				
"L." columella Say, 1817	8	2		MLP: 9234 (RM)
L. diaphana King, 1830	8	5	4	MLP: 9245 (RM)
L. pictonica Rochebrune & Mabille, 1885	1	3		MLP: 9253 (RM)
L. plicata Hylton Scott, 1953	3			MLP: 11264 (TM)
L. viatrix (d'Orbigny, 1835)	19	30	11	MLP: 11266 (TM)

Remarks: Castellanos & Landoni (1981) published a synonymic list of this family. There is no agreement among specialists with respect to the generic location of "*L.*" *columella*: Paraense (1983) places it in that genus whereas Hylton Scott (1953), Walter (1968), Castellanos & Landoni (1981), and Rudolph (1983) have it as *Pseudosuccinea columella*.

Planorbidae Rafinesque, 1815	MLP	MACN	FML	Reference Lot
Gen. *Antillorbis* Harry & Hubendick, 1963				
A. nordestensis (Lucena, 1954)	8			MLP: 7015 (RM)
Gen. *Acrorbis* Odhner, 1937				
A. petricola Odhner, 1937	8			MLP: 5089 (RM)
Gen. *Biomphalaria* Preston, 1910				
B. intermedia (Paraense & Deslandes, 1962)	*			
B. occidentalis Paraense, 1981	1			MLP: 7162 (RM)
B. oligoza Paraense, 1974	1			MLP: 11956 (RM)
B. orbignyi Paraense, 1975	5			MLP: 2261 (RM)
B. peregrina (d'Orbigny, 1835)	55	94	12	MLP: 780 (RM)
B. straminea (Dunker, 1848)	17	9		MLP: 7850 (RM)
B. tenagophila (d'Orbigny, 1835)	66	21	8	MLP: 350 (RM)
Gen. *Drepanotrema* Fischer & Crosse, 1880				
D. anatinum (d'Orbigny, 1835)	13	1		MLP: 4441 (RM)
D. cimex (Moricand, 1839)	13			MLP: 6757 (RM)
D. depressissimun (Moricand, 1839)	4	2		MLP: 5324 (RM)
D. heloicum (d'Orbigny, 1835)	3	1		MLP: 8534 (RM)
D. kermatoides (d'Orbigny, 1835)	21	18		MLP: 2136 (RM)
D. lucidum (Pfeiffer, 1839)	20	1		MLP: 7004 (RM)

Remarks: Rumi (1991) prepared a synonymic list of this family. For Apipé Falls in Corrientes Province, the presence of *Acrorbis sp.* (Rumi, 1986) was recorded. Lot MLP 5089 corresponds to the syntype of *Acrorbis odhneri*, a species is now synonymized with *A. petricola*.

Ancylidae Rafinesque, 1815	MLP	MACN	FML	Reference Lot
Gen. *Anisancylus* Pilsbry, 1924				
A. obliquus (Broderip & G. B. Sowerby I, 1832)	5			MLP: 2154 (RM)
Gen. *Gundlachia* Pfeiffer, 1849				
G. ticaga (Marcus & Marcus, 1962)	*			
Gen. *Hebetancylus* Pilsbry, 1913				
H. moricandi (d'Orbigny, 1837)	15			MLP: 3610 (RM)
Gen. *Laevapex* Walker, 1903				
Laevapex sp.	*			
Gen. *Uncancylus* Pilsbry, 1913				
U. concentricus (d'Orbigny, 1835)	26			MLP: 2153 (RM)

Remarks: Barbosa dos Santos (2003) was followed. Fernández (1981) published the synonymic list of this family. We considered Ancylidae as a family, although Bouchet & Rocroi (2005) include the tribu Ancylini into Planorbidae.

Physidae Fitzinger, 1833	MLP	MACN	FML	Reference Lot
Gen. *Physa* Draparnaud, 1801				
P. aspii Holmerg, 1909		1		MACN: 1407 (RM)
P. loosi Holmerg, 1909		1		MACN: 1404 (RM)
Gen. *Physella* Haldeman, 1843				
"P. cubensis"	12	2		MACN: 32605 (RM)
"P." venustula (Gould, 1848)	*			
Gen. *Stenophysa* Martens, 1898				
"S." marmorata (Guilding, 1828)	19	65	7	MLP: 7193 (RM)

Remarks: This family is still the object of important systematic revisions. According to the revision of Taylor (2003), *P. loosi* and *P. aspii* would be categorized as *incertae sedis*. Taylor (2003) placed *Physa acuta* Draparnaud, 1805, *P. cubensis* (Pfeiffer, 1839), and *P. venustula* in the genus *Haitia* Clench & Aguayo, 1932, on the basis of the absence of gland tissue in the sheath of the penis and considered that the species present in Argentina is *Haitia acuta* or *Haitia mexicana* (Philippi, 1841). However, Paraense & Pointier (2003) considered *P. cubensis* to be a synonym of *P. acuta.* Taylor (2003, 2004) pointed out in addition that the distribution of "*S. marmorata*" would not reach the Argentine territory and the specimens from Argentina could belong to other entities not assigned to any generic or specific category. Thus, it was decided not to innovate in the denomination of the referred or type material until the question is solved, which does not imply an agreement with such denomination.

RESEARCH NOTES

MALACOLOGIA, 2006, 49(1): 211–215

TWO NEW *HIRTUDISCUS* SPECIES FROM COLOMBIA (GASTROPODA: SCOLODONTIDAE)

Bernhard Hausdorf[1]* & Clara I. Medina Bermúdez[2]

ABSTRACT

Two new *Hirtudiscus* species are described from Colombia. ***Hirtudiscus triserialis*** n. sp. from the Departamento Cundinamarca is characterized by a large shell with three rows of up to 1 mm long hairs at the periphery and a distinctly sunken spire. ***Hirtudiscus antiochiensis*** n. sp. from the Departamento Antiochia is characterized by a moderately large shell with moderately dense growth striae and hairs up to 0.5 mm long.

Key words: *Hirtudiscus*, Scolodontidae, systematics, Colombia.

INTRODUCTION

Hylton Scott (1973) described the genus *Hirtudiscus* and classified it with the Endodontidae Pilsbry, 1895 (Punctoidea), on the basis of a single shell from the Cordillera Oriental in Colombia. Recently, Hausdorf (2003) examined the anatomy of a species of this genus and showed that it belongs to the Scolodontidae H. B. Baker, 1925 (= Systrophiidae Thiele, 1926), and is closely related to *Drepanostomella* Bourguignat, 1889, from which it differs in the presence of hairs, an aulacopod foot, and the vaginal appendage (anatomical data for *Drepanostomella*: Ramírez, 1993). Hausdorf (2003) described three new *Hirtudiscus* species from the Cordillera Oriental in the Departamentos Cundinamarca and Boyacá and the Distrito Especial. In the following account, two new species are described from the Departamentos Cundinamarca and Antioquia.

MATERIALS AND METHODS

Radulae were prepared by dissolving the dissected buccal mass in 5% potassium hydroxide for several hours. Then they were cleaned in an ultrasonic cleaner for 10 s and washed with distilled water. The cleaned radula was mounted on a small piece of coverslip in water and allowed to dry directly onto the glass. The coverslip was fixed to a SEM stub using double-sided tape and sputtered with gold in a Sputter Coater (GEA004S). The radulae were examined in a Leo 1525 scanning electron microscope.

The counting of the shell whorls (exactness 0.25) follows Kerney & Cameron (1979: 13). Shells were photographed without coating in a variable pressure scanning electron microscope (Leo 1455VP). The terms proximal and distal refer to the position in relation to the gonad.

The material on which this study is based is kept in the collection of the Universidad Militar Nueva Granada, Santafé de Bogotá (UMNG-MT), and the Zoologisches Museum der Universität Hamburg (ZMH).

Additional abbreviations: D = shell diameter; D3 = shell diameter at 3 whorls; H = shell height; leg. = collected by.

SYSTEMATICS

Scolodontidae H. B. Baker, 1925

Hirtudiscus Hylton Scott, 1973

Hirtudiscus Hylton Scott, 1973: 128. Type species (by original designation): *Hirtudiscus hirtus* Hylton Scott, 1973.

Hirtudiscus triserialis n. sp.

Figures 1, 3, 4, 7–10

Type Material

Holotype: Colombia, Departamento Cundinamarca: Parque Natural Chicaque near

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Soacha, Andean forest near Cascada, 2,400 m altitude, 04°41’31”N, 74°15’08”W (ZMH 37575, leg. C. I. Medina 19.10.2004, measurements: D = 11.5 mm, H = 4.3 mm).

Paratypes: Colombia, Departamento Cundinamarca: Parque Natural Chicaque near Soacha, Andean forest near river, 2,320 m altitude, 04°41’13”N, 74°15’37”W (UMNG-MT 301/1); Parque Natural Chicaque near Soacha, Andean forest near Cascada, 2,400 m altitude, 04°41’31”N, 74°15’08”W (UMNG-MT 302/12; ZMH 37576/4).

Type Locality

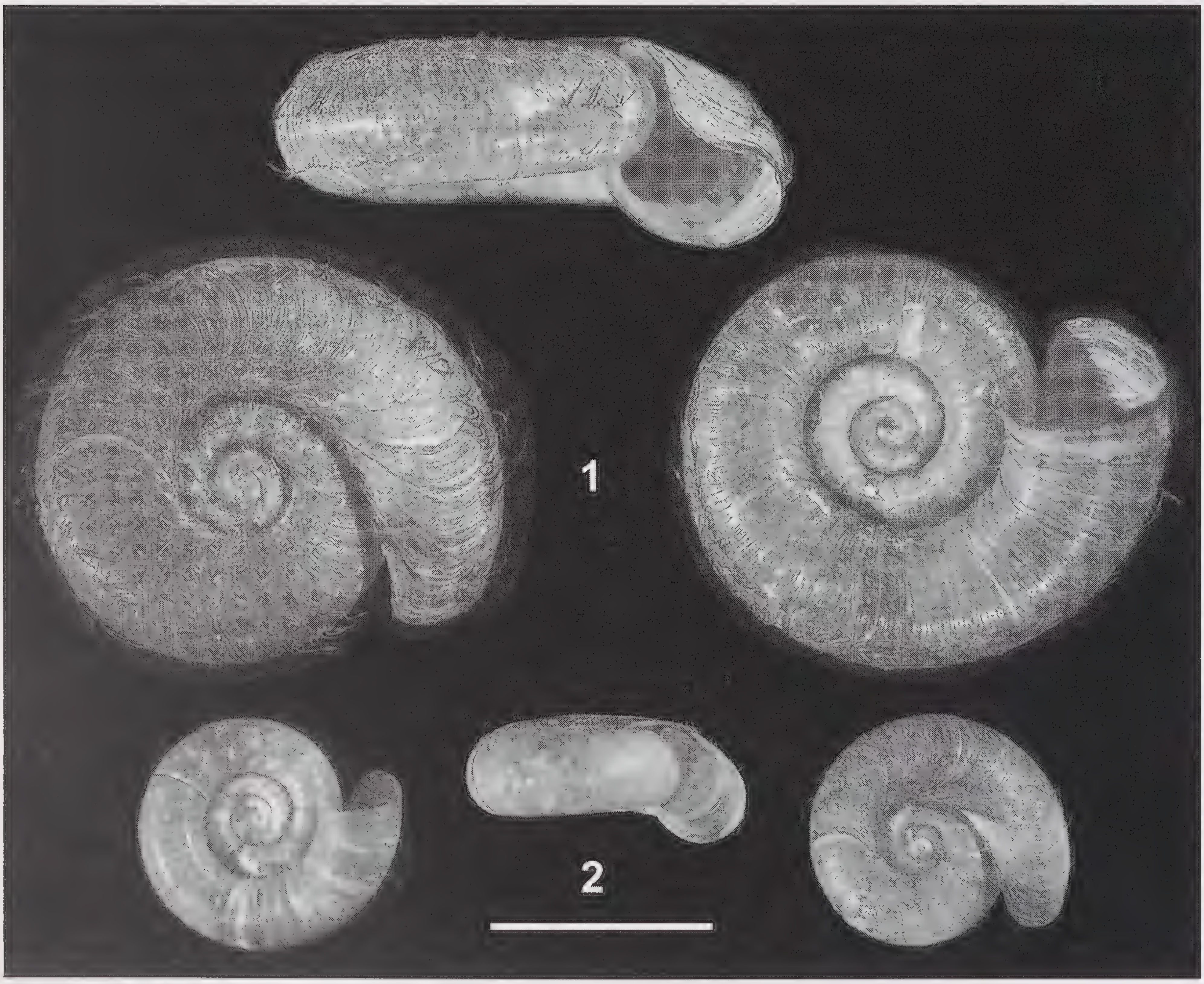
Colombia, Departamento Cundinamarca: Parque Natural Chicaque near Soacha, Andean forest near Cascada, 2,400 m altitude, 04°41’31”N, 74°15’08”W.

Diagnosis

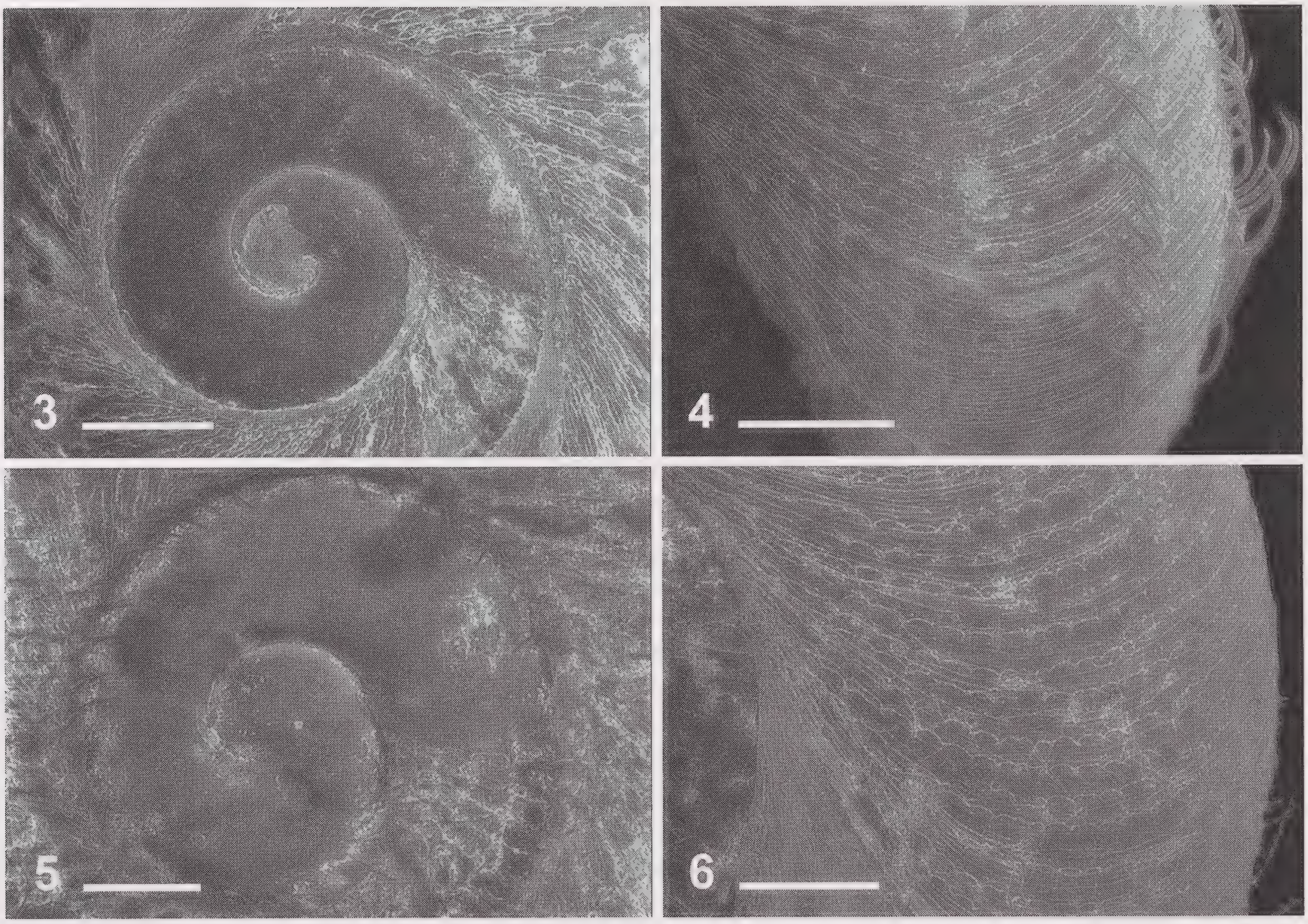
Hirtudiscus triserialis is characterized by a large shell (diameter 9.3–11.9 mm) with three rows of long hairs (up to 1 mm long) at the periphery and a distinctly sunken spire. Its genitalia differ from those of *H. comatus* Hausdorf, 2003, the only other anatomically known *Hirtudiscus* species, in the longer penis, the subapical insertion of the vas deferens on the epiphallus, and the globular swelling at the base of the peduncle of the bursa copulatrix.

Description

Shell (Figs. 1, 3, 4) discoidal; spire distinctly sunken; with 3.5–3.75 convex whorls; protoconch with indistinct spiral striae; teleoconch



FIGS. 1, 2. *Hirtudiscus* spp. Shells. FIG. 1. ***Hirtudiscus triserialis*** n. sp., Colombia, Departamento Cundinamarca: Parque Natural Chicaque, near Cascada (Holotype ZMH 37575). Scale bar = 1 mm; FIG. 2. ***Hirtudiscus antioquiensis*** n. sp. Colombia, Departamento Antioquia: La Ceja, Finca Guaycoral (Holotype ZMH 37577). Scale bar = 1 mm.



FIGS. 3–6. *Hirtudiscus* spp. Sculpture. FIGS. 3, 4. ***Hirtudiscus triserialis*** n. sp., Colombia, Departamento Cundinamarca: Parque Natural Chicaque, near Cascada (Paratype ZMH 37576); FIG. 3. Protoconch. Scale bar = 0.5 mm; FIG. 4. Shell sculpture on body whorl. Scale bar = 1 mm; FIGS. 5, 6. ***Hirtudiscus antioquiensis*** n. sp. Colombia, Departamento Antioquia: La Ceja, Finca Guaycoral (Holotype ZMH 37577); FIG. 5. Protoconch. Scale bar = 0.2 mm; FIG. 6. Shell sculpture on body whorl. Scale bar = 0.5 mm.

with three rows of long hairs (up to 1.0 mm) at, above and below the periphery, at the upper side there are several additional spiral rows of short extensions of the growth striae, of which those close to suture form short hairs, at the bottom side there are also a few spiral rows of short hairs close to suture; brownish-corneus; body whorl rounded; aperture oblique oval; upper insertion of the peristome not descending towards aperture, strongly bent backwards and, close to suture, abruptly bent downwards; thus, suture deeply impressed; peristome sharp, neither expanded nor thickened; umbilicus very wide, taking about 37–41% of the shell diameter.

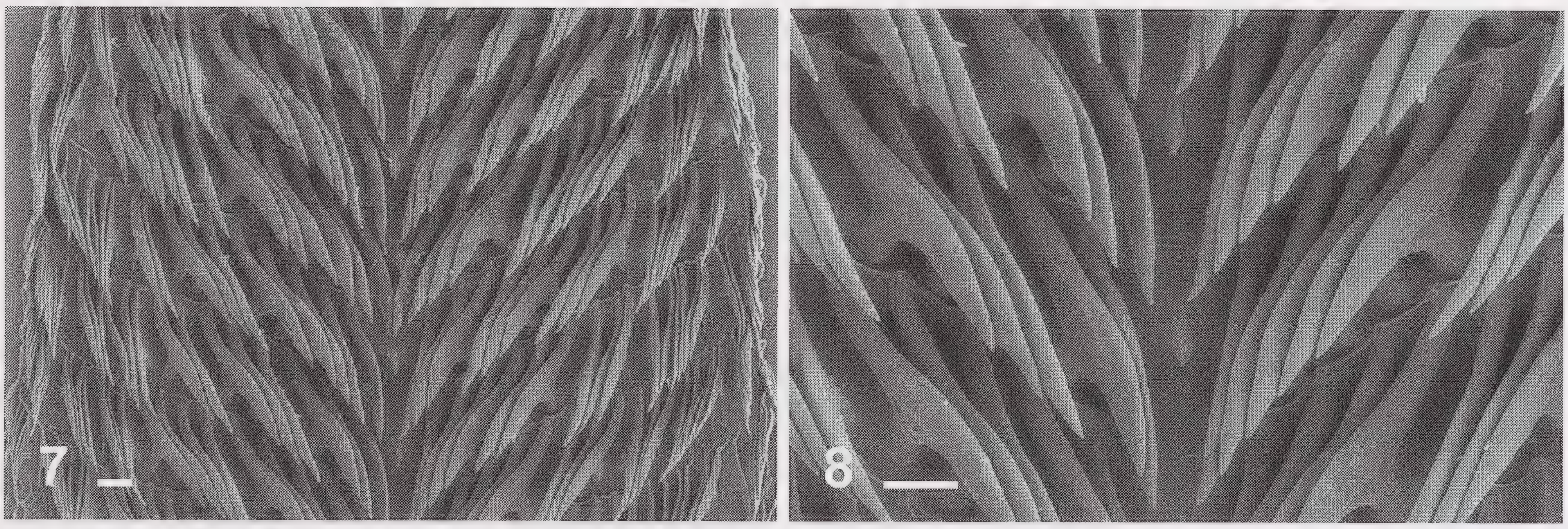
Measurements. Chicaque (n = 10): D: 9.3–11.9 mm, ø = 11.0 ± 0.7 mm; D3: 7.4–7.8 mm, ø = 7.7 ± 0.1 mm; H: 3.5–4.6 mm, ø = 4.1 ± 0.3 mm; D/H: 2.46–2.89, ø = 2.69 ± 0.14.

Anatomy (Figs. 7–10). Two specimens (ZMH 37576) were examined. The animal is yellowish. The foot is aulacopod. There is no distinct caudal pit or caudal horn at the tail, which is also not distinctly truncated. The sole is not distinctly divided. There are no distinct lappets at the mantle collar.

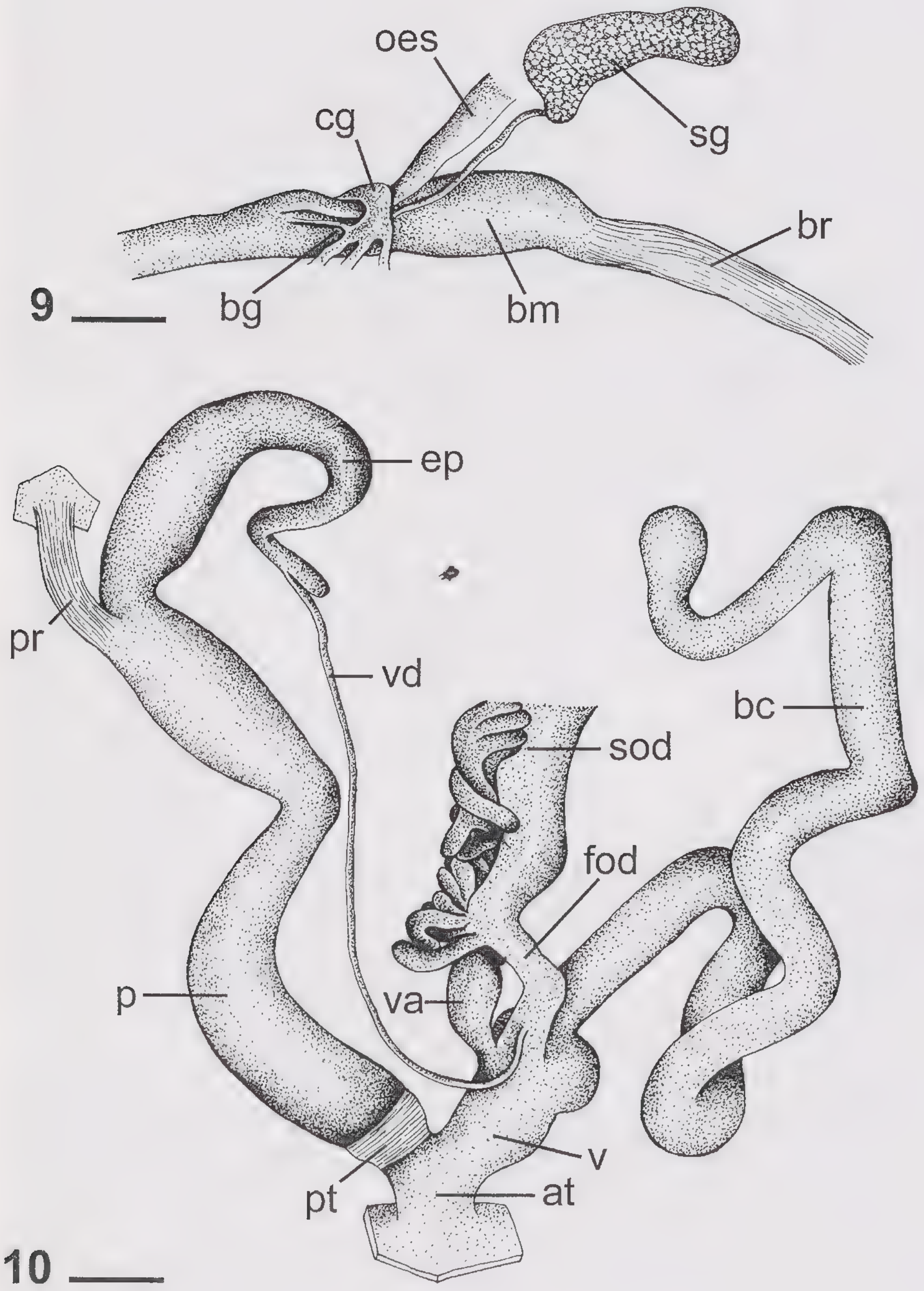
The buccal mass (Fig. 9) is extraordinarily elongated. The oesophagus and the salivary glands insert at about one-third of the length of the buccal mass. The tongue-shaped salivary glands are about half as long as the buccal mass. No jaw was found. The odontophore is restricted to the posterior third of the buccal mass.

The radula (Fig. 7) is long and narrow, with minute, narrow central teeth (Fig. 8) and 24 or 25 dagger-like lateral teeth on each side in each V-shaped row. The slightly curved cusps of the lateral teeth point toward the centre and are connected by an extension to the basal plates which point away from the centre. First lateral tooth is about as large as the adjacent laterals.

Genitalia (Fig. 10). The penis is long. Its middle section is slightly constricted. There is



FIGS. 7, 8. ***Hirtudiscus triserialis*** n. sp., Colombia, Departamento Cundinamarca: Parque Natural Chicaque, near Cascada (Paratype ZMH 37576). FIG. 7. Radula. Scale bar = 20 µm; FIG. 8. Central part of the radula with central teeth and first lateral teeth. Scale bar = 20 µm.



FIGS. 9, 10. ***Hirtudiscus triserialis*** n. sp. Colombia, Departamento Cundinamarca: Parque Natural Chicaque, near Cascada (Paratype ZMH 37576). Scale bar = 1 mm. FIG. 9. Buccal mass; FIG. 10. Genitalia. Abbreviations: at, atrium; bc, bursa copulatrix; bg, buccal ganglion; bm, buccal mass; br, buccal retractor; cg, cerebral ganglion; ep, epiphallus; fod, free oviduct; p, penis; pr, penial retractor; pt, penial tunica; oes, oesophagus; sg, salivary gland; sod, spermoviduct; v, vagina; va, vaginal appendage; vd, vas deferens.

a short penial tunica around the base of the penis. There is a large, wrinkled fold inside the penis. The wall of the penis is covered with numerous papillae with recurved, corneous hooks. The penis retractor inserts near the proximal end of the penis and on the diaphragm. The long epiphallus is distally broad and tapers towards its proximal end. The thin vas deferens inserts subapically on the epiphallus; it does not run through the penial tunica. The vagina is short. There is a club-shaped appendage at the vagina. There is a globular swelling at the base of the very long peduncle of the bursa copulatrix. The small bursa of bursa copulatrix reaches the digestive gland. The free oviduct is very short. The albumen gland is small. The right ommatophoral retractor runs between penis and vagina. In the second examined specimen, there are nine differently developed eggs with embryos in the spermoviduct. The distal genitalia, especially the male copulatory organs, are reduced compared with the specimen without eggs in the spermoviduct and appear as in subadult specimens.

Distribution

Hirtudiscus triserialis is known only from Andean forests at 2,300–2,400 m altitude in the Parque Natural Chicaque near Soacha in the Departamento Cundinamarca, Colombia.

Etymology

The specific epithet refers to the three rows of long hairs at the peripery of the shell (lat. *triserialis* = tree-rowed).

Hirtudiscus antioquiensis n. sp.

Figures 2, 5, 6

Type Material

Holotype: Colombia, Departamento Antioquia: La Ceja, Finca Guaycoral, Andean forest, 2,720 m altitude, 06°05'20"N, 75°23'35"W (ZMH 37577, leg. C. I. Medina, 05.12.2001, measurements: D = 5.7 mm, H = 2.4 mm).

Paratypes: Colombia, Departamento Antioquia: La Ceja, Finca Guaycoral, Andean forest, 2,720 m altitude, 06°05'20"N 75°23'35"W (UMNG-MT 303/4; ZMH 37578/1); La Ceja, Finca Guaycoral, Andean forest, 2700 m altitude, 06°05'21"N, 75°23'37"W (UMNG-MT 304/2).

Type Locality

Colombia, Departamento Antioquia: La Ceja, Finca Guaycoral, Andean forest, 2,720 m altitude, 06°05'20"N, 75°23'35"W.

Diagnosis

Hirtudiscus antioquiensis is characterized by a moderately large shell (diameter 5.2–6.0 mm) with moderately dense growth striae and hairs up to 0.5 mm long.

Description

Shell (Figs. 2, 5, 6) discoidal; spire sunken; with 3.25–3.5 convex whorls; protoconch with indistinct spiral striae; teleoconch with moderately dense, fine growth striae carrying up to 0.5 mm long hairs in spiral rows; brownish-corneus; body whorl rounded; aperture almost circular; upper insertion of peristome not descending towards aperture, strongly bent backwards and, close to suture, abruptly bent downwards; thus, suture deeply impressed; peristome sharp, neither expanded nor thickened; umbilicus very wide, taking about 37–44% of shell diameter.

Measurements. La Ceja, Finca Guaycoral (n = 6): D: 5.2–6.0 mm, ø = 5.8 ± 0.3 mm; D3 = 4.0–5.2 mm, ø = 4.6 ± 0.4 mm; H: 2.1–2.6 mm, ø = 2.4 ± 0.2 mm; D/H: 2.31–2.52, ø = 2.41 ± 0.08.

Remarks

Hirtudiscus antioquiensis differs from ***H. triserialis*** in the smaller shell and the lack of the three rows of very long hairs. However, its hairs are longer than those of the other known *Hirtudiscus* species. Moreover, it differs from *H. comatus* Hausdorf, 2003, in the more rapidly increasing whorls (in *H. comatus* the spire takes 45–56% of the shell diameter, in ***H. antioquiensis*** 31–39%; D3 in *H. comatus* 3.1–3.8 mm, in ***H. antioquiensis*** 4.0–5.2 mm), from *H. boyacensis* Hausdorf, 2003, and *H. curei* Hausdorf, 2003, in the larger shell with a sunken spire and less dense growth striae with hairs and from *H. hirtus* Hylton Scott, 1973, in the lack of distinct spiral striae on the protoconch and also in the larger shell with less dense growth striae with hairs.

Distribution

Hirtudiscus antioquiensis is known only from an Andean forest at 2,700 m altitude near La Ceja in the Departamento Antioquia, Colombia.

Etymology

The specific epithet refers to the occurrence of the species in the Colombian Departamento Antioquia (*antioquiensis* used as an adjective).

ACKNOWLEDGEMENTS

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Revised ms. accepted 17 February 2006

MALACOLOGIA, 2006, 49(1): 217–221

EFFECT OF AERIAL EXPOSURE ON PHYSIOLOGICAL CONDITION AND SURVIVAL OF *DIPLODON CHILENSIS* (BIVALVIA: HYRIIDAE) DURING TRANSLOCATION

Santiago Peredo[1]*, Esperanza Parada[1], Iván Valdebenito[2] & Matías Peredo[3]

INTRODUCTION

Freshwater mussels are an important component of the aquatic biodiversity of Chile, especially in the southern part of the country, and other geographical areas of the northern and southern hemispheres. The most common species in Chile is *Diplodon chilensis* (Gray, 1828), which is a unique species of Hyriidae with a distribution extending from 34°58'S to 72°48'S in both lentic and lotic environments in numerous hydrographic basins (Parada & Peredo, 2002). It is also present in Argentina between parallels 32°52' and 45°51'S (Bonetto, 1973). *Diplodon chilensis* populations have declined or been extirpated in lotic environments due to disturbance or habitat degradation from anthropogenic activity, mainly engineering projects. To date there have been no proposals in Chile for the protection of freshwater mussels. Relocation of unionids is being used as a conservation and management tool (Cope & Waller, 1995). They reviewed 33 projects and reported a mean survival of 50%, suggesting that relocation had been stressful to mussels. Furthermore, most relocations were monitored for < 1 year, and < 20% were monitored for five years. The success of most relocation projects has been predominately judged by mussel survival. Few studies examined measures such as growth, recruitment or condition index.

Peredo et al. (2005), studying relocation of *D. chilensis* in Chile and its long-term evaluation, reported positive results due to recruitment and recovery of mussels 18 years after relocation. However, little is known of the effect of handling and aerial exposure on mussel survival or recommendations on the most appropriate season to conduct relocations. It is hypothesized that relocation of freshwater mussel populations is a suitable management and conservation strategy if the stress of handling is minimized and time of the year fits with the physiological condition of mussels, the period when reproductive stress is low and metabolic rate sufficient for reburrowing into substratum. The goal of the present study was to evaluate the effect of aerial exposure on survival and physiological condition of *Diplodon chilensis* at two different times of the year, through short-term relocation experiments.

MATERIAL AND METHODS

Study Site

Lake Colico is located at 39°07'S, 72°00'W, in the pre-Andes Mountains region. The lake is part of the Chilean northern-patagonian lakes called the Araucanian lakes and belonging to the Toltén River basin. It is pristine, oligotrophic and with low species diversity. In summer, its temperature ranges between 19.5°C and 21°C and in winter, between 9.2°C and 9.4°C. Its maximum depth is 250 m.

The fish farm channel receives water from the Curaco River, an outlet of Lake Colico. Mean water temperatures at the fish farm of the School of Aquaculture of the Catholic University of Temuco at Los Laureles (39°2'S, 72°12'W) range between 9.6°C in August (winter) and 18.9°C in February (summer).

In late fall-winter and spring-summer periods of 2003, *D. chilensis* were randomly sampled by scuba diving at 2 m depth from Lake Colico and transported during 20 min to the fish farm in aerated coolers of 40 x 25 x 35 cm with lake water, located at Los Laureles 30 km from Lake Colico. The mussels were placed in the fish farm channel, where the experiments were conducted.

The experiment design of fall-winter and spring-summer studies consisted of three aerial exposure treatments (2, 4, 8 h), with three replicates per treatment and respective controls,

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TABLE 1. Mean water temperature, relative humidity, air temperature, and air temperature changes during exposure treatments with mussels in winter and summer study periods.

Study Period	Lake T in °C	Channel T in °C	Exposure duration in hours	Relative Humidity in %	Air T initial in °C	Air T end in °C	Δ T in °C
Winter	10.1	9.8	2	91	6.5	7.3	0.8
			4	91	6.5	8.0	1.5
			8	91	6.5	12.5	6.0
Summer	14.0	14.0	2	73	12.5	17.0	4.5
			4	73	12.5	17.6	5.1
			8	70	12.5	22.1	9.6

which were not emersed. Each treatment and its respective control for fall-winter and spring-summer periods consisted of 75 mussels (25 per replicate), chosen at random from the total mussels collected, and placed in wire cages (30 x 20 x15 cm mesh) to prevent escapement and predation. The bottom of each cage was covered with the channel substratum, and the cages were marked for later identification.

Prior to placing mussels into the cages, individual valve lengths (mm, anterior to posterior) and wet weights (g) were measured to determine condition index, using the relation $CI = WTM/L^3 \times 100$ (WTM = wet total mass; L = valve length) adapted from Newton et al. (2001). Wet total mass corresponds to the total weight of the specimen (valve weight plus soft tissue weight). We used wet mass to avoid sacrifice of animals. Air temperature and relative humidity were recorded during the time of aerial exposure of the mussels.

Three months after relocation, mussels were examined for survival (live vs. dead) and physiological condition. Two-way ANCOVA was used to establish statistically significant differences between initial condition index (iCI) and final condition index (fCI) of each of the treatments and respective controls, between final condition index (fCI) of treatments and controls, and between final condition index (fCI) of treatments of winter and summer studies. Differences among treatments were judged significantly at $P < 0.05$ level.

RESULTS

Aerial Exposure Period

All mussels subjected to 2, 4, and 8 h of aerial exposure and the respective controls in the fall-winter and spring-summer periods survived the initial treatments. However, some mussels of all treatments showed signs of stress such as gaped valves and protruded feet. Evidence of stress in mussels was observed in experiments conducted in both periods of the year, with stress signs becoming more evident in mussels exposed for 8 h.

Water temperature of Lake Colico and that of the fish farm channel did not show significant differences in the experiments carried out concurrently. However, differences were observed between experiments performed at different periods of the year. Thus, lake water and channel water temperature in spring-summer period was 4.2°C higher than in fall-winter period (Table 1). Air temperature during the 8 h exposure treatment was higher in spring-summer period (9.6°C) than in fall-winter period (6.0°C) (Table 1).

Resurvey Period

After three months in the channel, mussels did not showed differences in survival between experiments carried out in the fall-winter period. Mussels did showed differences in treatments conducted in the spring-summer period (Fig. 1). In fall-winter period, survival was

TABLE 2. Values of p ANCOVA comparing initial Condition Index (iCI) and final Condition Index (fCI) of treatments and controls of winter and summer studies (p-value < 0.05).

fCI vs iCI	Winter p value	Summer p value
control	0.526	0.345
2h	0.13	0.984
4h	0.897	0.506
8h	0.841	0.764

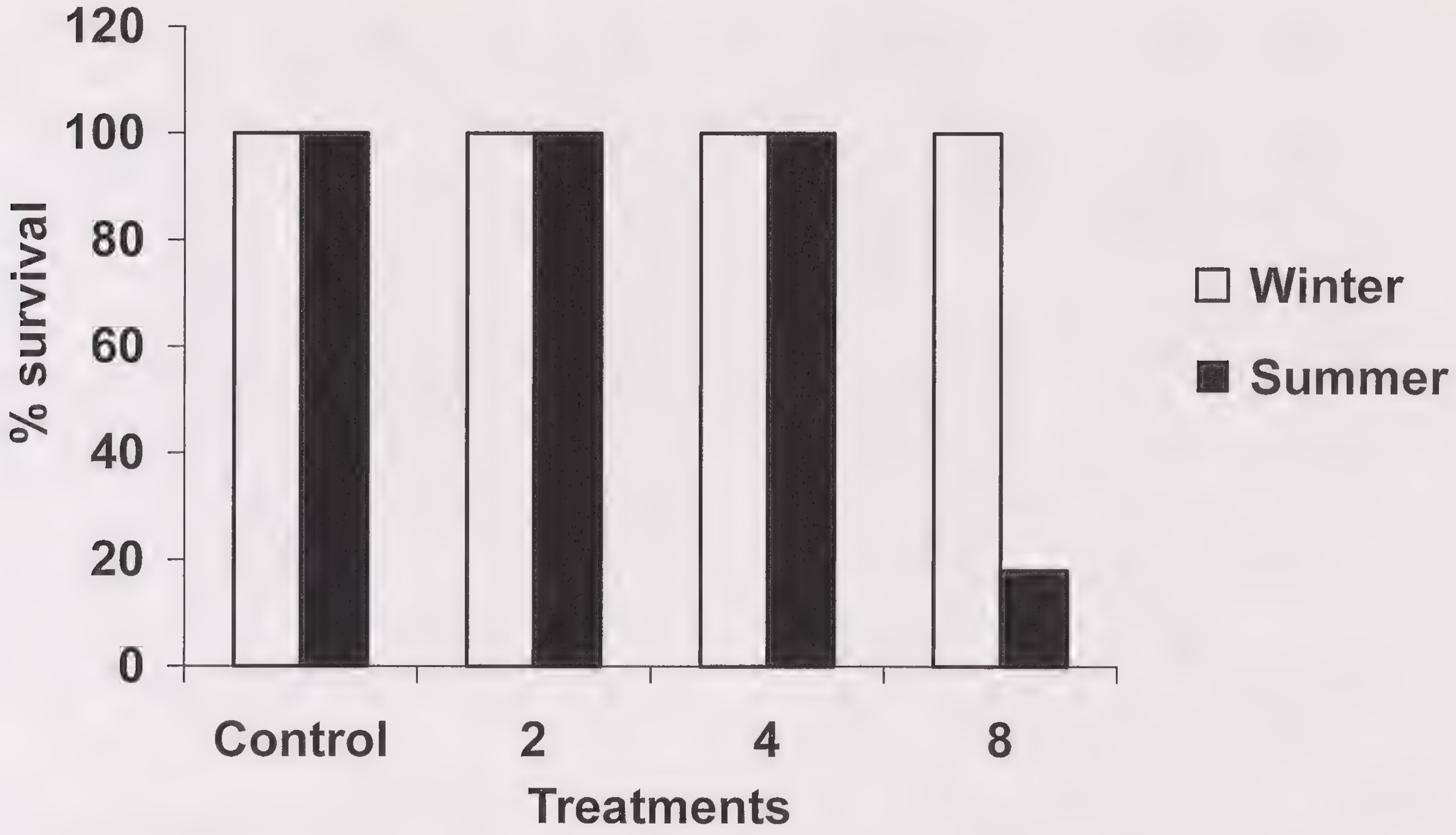

FIG. 1. Percent survival of 0 (control), 2, 4, and 8 hours emersion treatment groups of mussels of summer and winter study.

100% in each of the treatments (2, 4, and 8 h of air exposure) and the respective controls. In the spring-summer period, the 2 and 4 h aerial exposure treatments, survival was 100%, and 18% in 8 h air exposure treatment (Fig. 1). The control survival was 100%. In two of the replicates of the 8 h treatment, survival was 0% (survival was not observed in 100% of the mussels).

Two-way ANCOVA showed no significant differences between initial condition index (iCI) and final condition index (fCI) of each of the treatments and respective control (Fig. 2, Table 2), nor were there significant differences between final condition index (fCI) of treatments and controls, as well as between final condition index (fCI) of treatments of winter and summer studies (Tables 3, 4). In the spring-summer test we did not consider two replicas from 8 h treatment because the mussels were dead.

TABLE 3. Values of p ANCOVA comparing control and each of the treatment final Condition Index (fCI) of winter and summer studies (p-value < 0.05).

Treatment and respective controls fCI	Winter p value	Summer p value
2 h	0.492	0.871
4 h	0.541	0.602
8 h	0.568	0.506

DISCUSSION

The differences in survival observed in the winter experiment relative to the summer experiment is attributable to air temperature and relative humidity rather than to the duration of aerial exposure. The former factors contribute to dessication of mussels. The locality where the experiments were conducted has a mediterranean climate, determined by the high relative humidity present from April to November (Di Castri & Hajek, 1976). Studies carried out by Dietz (1974) Byrne & McMahon (1994), Waller et al. (1995), and Bartsch et al. (2000) corroborate our results.

Diplodon chilensis is a tachytictic species, being reproductively active during spring-summer, completing glochidial development in late summer (Peredo & Parada, 1986). These features could explain the survival differences in winter vs. summer. In summer, the mussels

TABLE 4. Comparison among treatment final Condition Index (fCI) of winter and summer studies (p-value < 0.05).

Treatment fCI	Winter p value	Summer p value
ICf 2h v/s ICf 4h	0.9	0.465
ICf 2h v/s ICf 8h	0.093	0.102
ICf 4h v/s ICf 8h	0.102	0.967

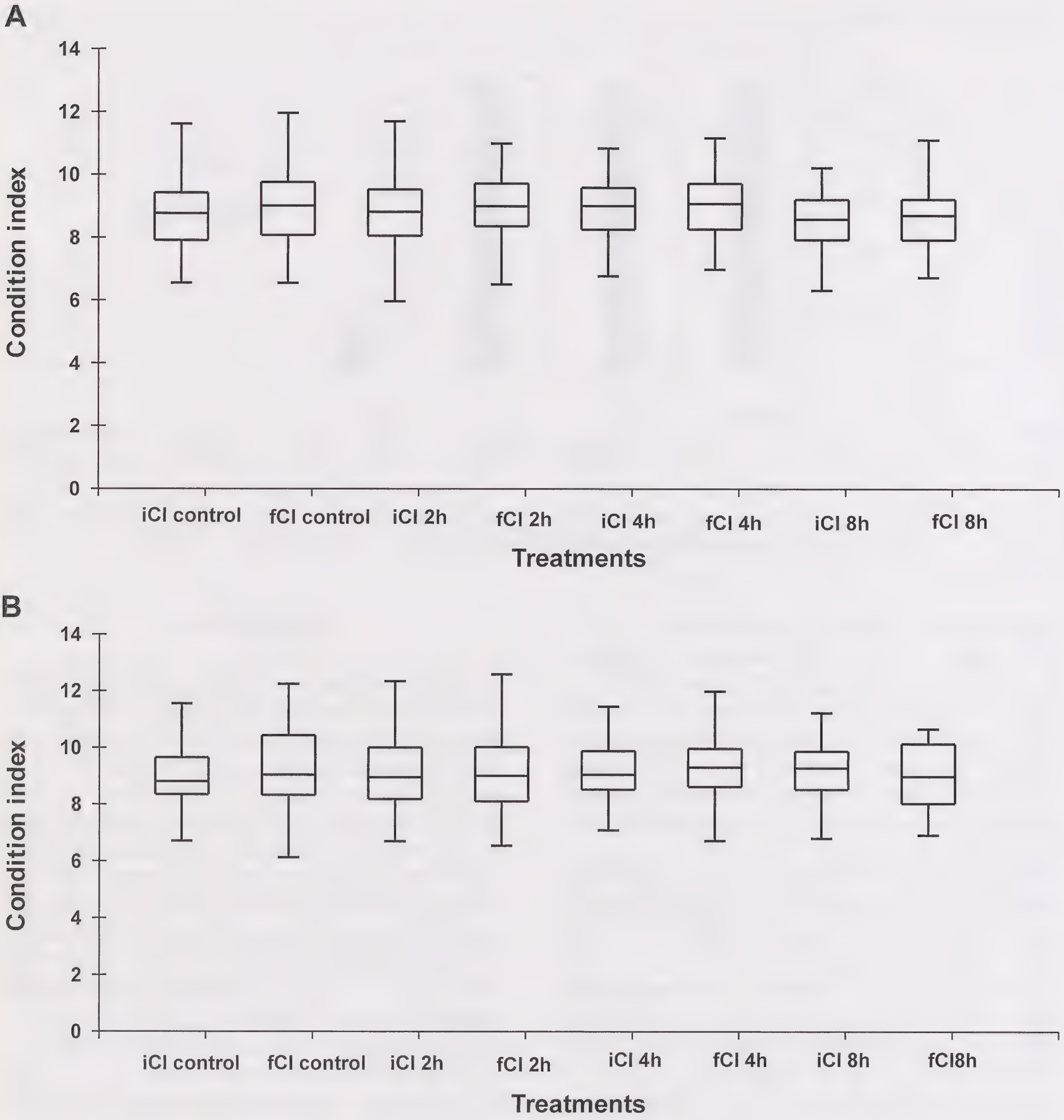

FIG. 2. Mean initial Condition Index (iCI) and final Condition Index (fCI) during 3 months of relocation into channel of mussels exposed to 0 (control), 2, 4, and 8 hour treatment (A = winter study; B = summer study).

would be more vulnerable to perturbation (aerial exposure) due to their reproductive condition, as was seen in individuals with gaping valves when exposed for 8 h and temperatures above 20°C. Conversely, winter low temperatures and high relative humidity would allow individuals to tolerate longer exposure periods. Similar results have been reported by Waller et al. (1995) in fall and spring for *Amblema plicata,* a tachytictic species.

With regard to the physiological condition of *D. chilensis* after three months in the fish farm channel, results show no significant differences between values of the CI before and after the aerial exposure, in survivors of the three treatments at the same time of year, neither between experiments conducted in winter versus summer. Specimens that survived the treatments retained their initial condition after relocation at any time of the year.

Our results coincide with those of Newton et al. (2001) for mussels relocated to a river.

In conclusion, we found that air temperature and relative humidity during emersion are the factors that most effect mussel survival and that after three months, and surviving mussels exhibited a physiological condition similar to that recorded before relocation. The most appropriate time to relocate *D. chilensis* populations is the fall-winter period, when temperature and relative humidity do not significantly affect survival. The results of our study provide baseline information for developing relocation guidelines for handling this species.

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LETTERS TO THE EDITOR

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CHECKLISTS, SYSTEMATICS AND THE CLECOM INITIATIVE: AN ALTERNATIVE VIEW FROM EUROPE

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INTRODUCTION

Davis (2004) raised important issues about the function and construction of species checklists, and, by way of examples, provided a critique of two recent lists produced in Europe: the CLECOM I list for the land and freshwater Mollusca of northern, Atlantic and central Europe (Falkner et al., 2001), and the more detailed list for France alone (Bouchet, 2002; Falkner et al., 2002). It may be noted that CLECOM I is accompanied by CLECOM sections I and II (Bank et al., 2001), which provides a supraspecific classification for a larger area, including the whole of Europe, Turkey and Macaronesia.

His critique provoked two responses from the authors (Bouchet, 2006; Bank et al., 2006). In the first of these, some differences of opinion are thought to reflect the impact of real differences in the degree and character of taxonomic differentiation in regional faunas on thought and practice, while in the second the authors suggest that Davis is privileging molecular taxonomy at the expense of the use of morphological characters. The reference by Davis (2004) to the notorious "Nouvelle École" of Bourguignat and his followers (Dance, 1970), in particular, is seen as provocative and unjustified. We note that a significant part of these arguments relate as much to the principles and procedures of classification as to the function and content of checklists as such.

Both Davis and his respondents raise general and more specific issues; the latter are mainly concerned with details of work on particular freshwater groups. As workers mainly on terrestrial molluscs, we do not intend to enter into those debates. We believe, however, that the general issues are of significance to the malacological community, and there is a danger that arguments about such lists could become very parochial, reinforcing the rather negative image that tarnishes taxonomists generally. At the extreme, they could provide ammunition to those wishing to overturn the whole Linnean system (Godfray & Knapp, 2004).

We believe that these checklists do indeed raise issues of general importance, and we attempt to identify and resolve them. While one of us (RADC) is both a user, whose tyranny Bouchet (2006) suspects, and also an Anglophone, believed to belong to a conservative group with "lumping" tendencies (criticisms made in both responses), we hope that our combined, international contribution will be seen as constructive.

THE EUROPEAN FAUNA AND THE CLECOM INITIATIVE

Before any direct engagement with the arguments, we should acknowledge that the compilation of a European checklist presents particular problems. Proportionately, there are far more early descriptions of species than in any other part of the world; these are often inadequate by modern standards. Language problems, and the limited circulation of many publications, have resulted in a considerable amount of accidental synonymy, aggravated by some extreme schools of taxonomic practice (e.g., the "Nouvelle École" referred to above). A variety of rival higher classifications exist; some, apparently adopted completely by CLECOM, are far from universally known or accepted (e.g., Shileyko, 1978, 1984). National checklists reflect local traditions and ways of thinking, and different names for the same species may co-exist in the literature for many decades. Hence, the CLECOM initiative, endorsed by Unitas Malacologica in 1995 (Bank et al., 2006), represents a brave and arduous attempt to achieve coherence and uniformity. It is clear, both from the account of procedure given in the original (Falkner et al., 2001) and in the response to Davis (Bank et al., 2006), that much time and effort was spent not only

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in critical examination of literature, but also in finding and examining types. Nor has there been a slavish adherence to priority; individual authors of the CLECOM lists have sought and obtained ICZN rulings suppressing the earliest, but scarcely used names for some species. Nevertheless, we find ourselves in agreement with many of Davis' concerns, and we have some others that we believe should be expressed openly.

CHECKLISTS AND CLASSIFICATION

Bouchet (2006) heads one section of his response "Regional species checklists: what they are not." In this, he agrees with Davis, and with us, that they are not appropriate vehicles for advancing novel phylogenetic hypotheses, and that "elaborate, finely dissected classifications" are unnecessary. CLECOM I, however, presents us with such a finely dissected classification, and, through the nomenclature involved, also accepts, without published references, phylogenetic hypotheses that are by no means universally accepted. A checklist is not the kind of publication in which taxonomic decisions are made, and should contain references to publications in which the decisions adopted were made and supported by evidence. In their replies to Davis, the authors frequently explain in detail why they have used particular names and classifications. Such explanations would be superfluous if the information was accessible through reference to the detailed monographs or descriptions used.

We acknowledge that this task of documentation, for such a large fauna, is a daunting one. It is easier for smaller regions, as shown in the last authoritative British checklist (Waldén, 1976), in which the new list is supported by notes explaining changes from previous lists, and gives details of ICZN opinions and lists in each case. Bank et al. (2006) comment on Cameron's (2003) use of the name *Oxyloma pfeifferi* (Rossmässler, 1835), rather than the earlier *O. elegans* (Risso, 1826), as an example of traditionalism. In the context, however, it was used on the basis of Waldén's explicit argument, to retain consistency. There is now no doubt, and a new British checklist (Anderson, 2005) acknowledges, that *elegans* is indeed the correct epithet; in the meantime, a secondary publication (an identification guide for British users) stuck to the locally accepted name, but also drew attention to the use of *elegans* elsewhere and in the CLECOM list. Similarly, the authoritative account of Polish land molluscs includes synonyms and taxonomic notes (Riedel, 1988). The CLECOM list offers no synonyms to assist users.

Furthermore, some entries in the CLECOM list evidently rest on the unpublished conclusions of the authors. In a few cases, this results from the examination of types, and of new material from type localities, originally described by members of the "Nouvelle École", no doubt accounting for the reference in the title of Davis' paper. Such entries sometimes revive old species names later synonymised with others. For example, in species in the genus *Euconulus*, old names are rescued from synonymy, and the number of species increased, on the basis of work in preparation that has not yet appeared. In this case, the fact that work is in preparation is made known not in the CLECOM I list itself, but in the list for the French fauna (Falkner et al., 2002), which is annotated. Those consulting the CLECOM I list alone have no guidance, nor can they tell what has happened to the generally recognised *E. alderi* (Gray, 1840), which disappears without trace. We do not doubt that such examinations were thorough; the conclusions may, indeed, be correct. The problem is that the evidence is not visible. Those in doubt about a particular identification are left to chase round museums to inspect the types for themselves, and, indeed, may remain unsure from which currently recognised species the segregation has been made. We note that the French list (Falkner et al., 2002) is annotated, and avoids some of these problems.

Put simply, checklists are a product of systematics, not substitutes for it. While it is true that traditional taxonomic revisions have become unfashionable (Wheeler, 2004), checklists depend on such revising monographs and on new descriptions or redescriptions of species. These tasks are hard, but not impossible; we would cite, for example, the work of Alan Solem on endodontoid snails of Pacific islands and camaenid snails in Australia, encompassing descriptions or revisions of over 600 species (Cameron et al., 2005). As Bouchet (2006) says "... checklists are only as good as the quality of the science behind them", a point also made by Davis. Of course, some science may be both good and wrong; more information, or new analytical techniques may alter conclusions. Changes require documentation.

Bouchet (2006) also expresses concern that the desire of users for stability inhibits the introduction of necessary and justified change; can users impose a "tyranny" that must be resisted if progress is to be made? It is true that users desire stability; few, however, are likely to insist on it at all costs. Within the European fauna, segregations of very closely related species have been accepted without complaint, and the new (or old, but resuscitated) names have been incorporated into national and local checklists. A particular irritation for users arises when both generic and trivial names are altered, without the integrity of the taxon itself being questioned. Trivial names alter as a result, usually, of differing local usages resolved by priority; generic alterations appear to many users to be whimsical, reflecting a new, and often short-lived phylogenetic hypothesis. Changes of this sort promote confusion; the classic paper on the ecology of British land molluscs (Boycott, 1934) still holds valuable insights, but is impenetrable to the uninitiated because of nomenclatural changes since. Of course, many of these changes are justified, but users are right to resent repeated changes at generic level. We consider problems of supraspecific nomenclature further below.

Bank et al. (2006) cite many European authors who have accepted the CLECOM checklist as the basis of their nomenclature. However, others have not, for example Wiktor (2004) for the Polish fauna, or Cameron (2003) for the British and Irish fauna. The new British and Irish checklist (Anderson, 2005) similarly adopts some, but not all of the changes introduced in CLECOM. Thus, it is clear that the aim of a common list (of course, subject to revision over time) has not been achieved, because basic disagreements between systematists, rather than between systematists and users, have not been resolved. We fear that some users adopt the list assuming it to be authoritative, aware of their own lack of expertise in taxonomic matters, but unaware of the disagreements that still exist among specialists.

In practical terms, we acknowledge that it is probably impossible to get all specialists on a regional fauna to agree, unreservedly, to a standard checklist. An extensive consultation beforehand, and a willingness to list areas of ambiguity or disagreement would increase the chances of "buy-in" by the professional community as a whole. The three of us engaged in systematic research (BMP, AR and AW) were not consulted in their areas of expertise. Areas of ambiguity and disagreement would also be minimised by omitting some non-mandatory levels in the hierarchy of classification.

SYSTEMATICS AND TAXONOMIC JUDGMENTS

As mentioned earlier, the debate over these checklists reflects a wider concern with systematics generally (Godfray & Knapp, 2004), prompted partly by the increasing volume of molecular studies, and partly by the parlous state of funding and respect for traditional alpha and revising taxonomy (Wheeler, 2004). It is clear, also, that a concern for the consequences in conservation policies is involved (Bouchet, 2006; Davis, 2004). It seems to us that the participants have, to some extent, talked past each other. CLECOM is not the "Nouvelle École" resurrected, nor does Davis give undisputed primacy to molecular data. Rather, the debate is between those who wish to recognise as much geographical variation, and as much detail of currently accepted phylogeny in nomenclature as possible, and those who prefer a more conservative approach, requiring more, and more conclusive evidence before making nomenclatural changes. Davis' approach to these problems is essentially that of the population biologist; populations of a species vary. The variation may be interesting in itself, but before it acquires nomenclatural significance, some marked discontinuities must be shown, and shown to be genetic. By inference (since the data are not presented in detail), the CLECOM approach is more typological, because although, no doubt, several (or many) specimens are examined, revision proceeds on the basis of perceived non-overlap in characters between some allopatric populations, without evidence of the range of variation to be found within each designated taxon being presented. Again, this distinction is not dependent on the use of molecular data; we again refer to Solem's monographs (Cameron et al., 2005), in which copious morphometric and anatomical data are presented and analysed. It could be said, in legal parlance, that CLECOM goes for "the balance of probabilities", Davis for "beyond all reasonable doubt".

In this particular respect, taxonomy differs from nearly all other aspects of biological science. A disproved theory elsewhere can simply disappear from view (other than for historians of the science). In taxonomy, nomenclatural acts become enshrined in the literature. Revision of a group is an arduous task of surveying early literature and searching out of type material, made harder by the accumulation of new or badly documented names.

Changes should be very well supported and documented, and we suggest that there are many occasions when the possibility that an existing classification is wrong could be raised in publication without rushing to change nomenclature. Given the "debris" of erroneous classifications, Davis' stance seems to us to be the more prudent.

All participants in this debate adhere, in principle, to the biological species concept. All accept, in principle, that taxa at any level should be monophyletic; we are all cladists now. All recognise that, in a majority of cases, current nomenclature neither rests on direct evidence of reproductive isolation, nor on molecular data alone. Species as described are essentially morphospecies (Eldredge & Cracraft, 1980). In this context, we draw attention to a significant feature of terrestrial gastropods: self-fertilization is widespread, and inadequately researched and documented (but see Jordaens et al., 1998, 2000, 2001; Reise et al., 2001; Nicklas & Hoffman, 1981). Where it is the norm, the biological species concept does not apply, and, inevitably, nomenclature rests on the degrees of difference between populations or forms, whether morphological or molecular. This feature of terrestrial molluscs may well lead to the conclusion that some sister species, living in sympatry, are in fact ecophenotypic variants of the same stock. The complexities of such situations can best be explored by molecular analysis (Armbruster, 1997).

It could be said that convergence and parallelism are the curses of cladistic and phylogenetic taxonomy (in contrast, they are blessings for the evolutionary biologist). We might add ecophenotypic variation as an additional burden for the taxonomist to bear; both some shell and some anatomical characters are known to vary substantially with environmental conditions and seasons (e.g., Solem & Christensen, 1984). While molecular techniques, even with the very sophisticated analytical software available, are not the only weapon in the modern taxonomist's armoury, they have been used, elegantly, to reveal the convergent nature of morphological characters previously believed to be apomorphies (Gittenberger & Schilthuizen, 1996). An even more complex picture is obtained in a recent study of *Mastus* species (Parmakelis et al., 2003), with molecular variation suggesting many episodes of population mixing, whereas morphology suggests strong selection varying with locality.

While species, therefore, are generally recognised to be real entities, their definition is frequently, of necessity, more arbitrary. For taxa above this rank, the only rule that commands general assent is that, to the best of our knowledge, they should be monophyletic. Demonstration that they are not is an accepted ground for revision. A quick survey of higher taxa used in European terrestrial and freshwater malacology reveals just how unstable and contentious they are. Convergence and parallelism are very frequent (Davis, 1979; Wiktor, 1984; Pokryszko, 1994), and differing suites of characters can yield different conclusions. We certainly agree with Bank et al. (2006) that molecular data are not the only source of reliability. A field badly neglected in non-marine molluscan taxonomy is ontogeny, perhaps because it is more demanding of time and patience than other sources. We know the details of life history for only a minute proportion of the European fauna, and there appears to be only one case in which patterns of development have been polarised for cladistic analysis (teeth in the shell-mouth; Pokryszko, 1997).

Given the unreliability or ambiguity of many characters used to define higher taxa, it seems prudent to go for relatively few, large and inclusive taxa that have a greater probability of remaining monophyletic in the face of new research, rather than attempting, through nomenclature, to capture virtually every branch point within a clade. The more subdivision, the more opportunities there are for error. This is not to deny the interest of detailed cladistic studies at family level and below, which have revealed patterns of evolutionary divergence that can be related to environmental history (e.g., Pokryszko, 1997; Douris et al., 1998; Wiktor, 2000). We are not persuaded that all steps in such radiations need to be entrenched in nomenclature. Biogeographical and evolutionary studies are based on monographs and cladograms, not checklists and names.

Another issue in the debate concerns subspecies. Classically, subspecies are seen as distinctive, geographically replacing forms of a species (O'Brien & Mayr, 1991). In practice, allopatric populations, with no contact, but definable differences, are given subspecific rank when the differences are not thought to be sufficient to justify species status, but the group as a whole is believed to be monophyletic. While we agree with Davis, and, indeed, with Bank et al. (2006), that some listed subspecies in checklists are dubious entities, their total elimination from all checklists seems unwarranted. Some may well turn out on further study to be worthy of species status, and reliable records of subspecies can then be allocated appropriately. It is worth noting, however, that if the term has been correctly applied (to allopatric or parapatric forms in a monophyletic group), recorders and naturalists will tend to name forms on their location, rather than on their characters, perhaps generating a spurious repeatability and coherence. In particular cases, where only one subspecies is known in the region for which a list is compiled, the subspecific epithet can well be omitted (Anderson, 2005). Given the very variable quality of subspecific designations, it seems rash to use the category as the basic unit of recording. That CLECOM I does so reflects a deep concern with the effect of nomenclature on conservation policies, the issue on which its authors and Davis disagree most strongly.

CHECKLISTS, TAXONOMY AND CONSERVATION

Davis (2004) singles out for criticism the explicit statements in Falkner et al. (2001) and in Bouchet (2002) supporting the use of subspecific names, even where the evidence does not unequivocally comply with the conventional definition of that rank, on the grounds that named entities are necessary if conservation campaigning is to be successful, and conservation policy to be implemented. There is much pragmatic force in this argument; names reify, creating a unique object for consideration. Bouchet (2006) emphasises this point, learnt by long experience in fighting battles for conservation. The complex, often mathematical, descriptions of within and between population variation that characterise the evolutionary, population genetics approach do not lend themselves easily to sharp campaigning or to legislative decisions.

Nevertheless, we agree with Davis that this is a distortion of taxonomic practice; in his words "… it risks to reduce taxonomy to a mere artifice possibly with grim consequences". The consequences Davis mentions are concerned with an explosion of badly supported names, confusion and taxonomic inflation. We see a significant further danger. Rightly, Bouchet (2006) says that we do not operate in a sociological vacuum. But we do not operate in a legal or political vacuum either. Those whose interests are threatened by specific conservation measures will quickly exploit a perceived inflation of taxonomic entities. Forensic examination of expert witnesses, and of the assumptions on which their conclusions were based, would reveal the underlying confusion, making the case for conservation harder. If the science underlying one nomenclatural decision is found wanting, other such decisions based on better evidence will share in the discredit. Other arguments, however difficult, should be used to advance conservation policies, and we should stick to strictly scientific criteria for nomenclatural decisions.

Despite our criticisms of the CLECOM list as it stands, we certainly support the intentions of the authors. For reasons given at the start, Europe has particular need of an agreed list of land and freshwater molluscs. The fact that the list has initiated a fierce debate within and outside that continent indicates its heuristic value. That it has exposed differences of view both about taxonomic practice, and about the functions and form of checklists can only be beneficial.

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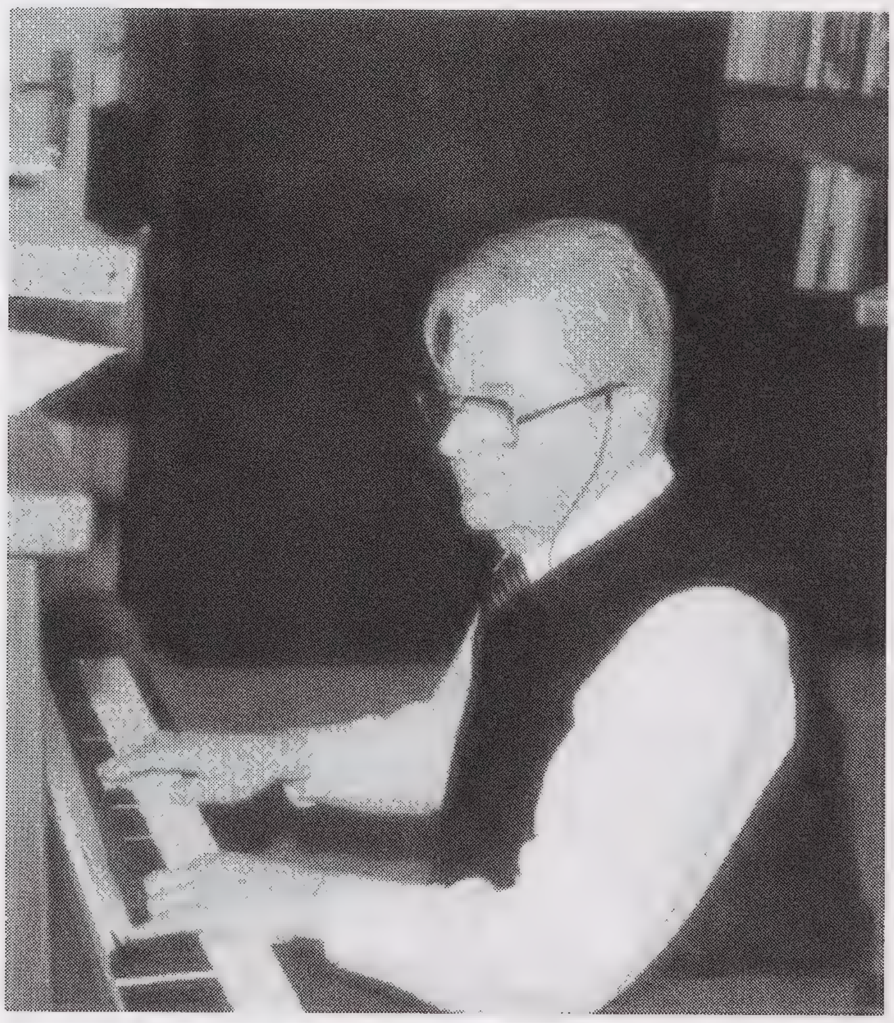
ADOLF M. ZILCH, 1911–2006



Dr. Adolf M. Zilch (Foto: Dr. H. Schütt, 1979)

Malacologia honors the life of Dr. Adolf Michael Zilch, who passed away on 1 January 2006 at the age of 94. He was a giant in the field of malacology and a marvelous friend to those of us who knew him well. He served *Malacologia* since the inception of the journal.

Dr. Zilch was one of the most recognized malacologists worldwide. With his handbook on the "Euthyneura", which is a standard tool even today nearly 50 years after its publication, he gained high international reputation. His vast knowledge in the field of world-wide terrestrial malacology became famous. A. Zilch was one of the founders of the Unitas Malacologica and for many decades president of the German Malacological Society. At the research institute Senckenberg, Adolf Zilch held the position of the curator of the mollusc collection for more than 40 years. He was indeed the curator's curator. He served as vice-director of the institute and head of the department of zoology as well as editor of three scientific journals, among them the *Archiv für Molluskenkunde*. After retirement in 1976, A. Zilch still worked in the malacological section for another 20 years as honorary collaborator. Besides malacology, his interests focused on music and history, and he was a highly recognized specialist in the field of numismatics.

G.M. Davis, Editor-in-Chief

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INVENTORIZING AN INVADER: ANNOTATED TYPE CATALOGUE OF CORBICULIDAE GRAY, 1847 (BIVALVIA, HETERODONTA, VENEROIDEA), INCLUDING OLD WORLD LIMNIC *CORBICULA* IN THE NATURAL HISTORY MUSEUM BERLIN[1]

Matthias Glaubrecht[2]*, Zoltan Fehér[3] & Frank Köhler[2]

ABSTRACT

The Corbiculidae Gray, 1847, is a group of limnic clams of considerable ecological and evolutionary potential. Among them, some freshwater species of *Corbicula* Megerle von Mühlfeld, 1811, are hyper-invasive neozoon in North and South America as well as Europe where currently some taxa are rapidly extending their range rendering them of special ecological importance. Next to the extraordinary invasive potential of the "Asiatic clam", corbiculids are remarkable in their recently discovered wide spectrum of reproductive strategies, including intrabranchial incubation (brooding). In concert with their colonization ability, these biological properties render corbiculids an ideal model group for studying evolutionary transformations. However, the taxonomy of these clams is in a chaotic state, with phylogenetics being largely unresolved. As a first step towards a comprehensive systematic revision of Corbiculidae, we here document the type material in the Natural History Museum of the Humboldt University in Berlin (ZMB), comprising essentially the invasive *Corbicula* but supplemented by those taxa traditionally attributed to *Batissa* Gray, 1853, and other genera. This account adds considerably to the compilation by Counts (1991), who omitted almost all ZMB material of *Corbicula*. We report on a total of 45 types (33 for *Corbicula,* 8 for *Batissa,* 1 for *Polymesoda*, and 3 of unresolved generic affinity) represented in the ZMB; this reflects more than 7% of nominal taxa known in corbiculids.

Key words: taxonomy, freshwater clams, *Batissa*, invasive species, lectotype designation.

INTRODUCTION

The Corbiculidae Gray, 1847, is a group of brackish-water and freshwater clams of large ecological and evolutionary potential. This widespread family contains moderate-sized bivalves with characteristic concentrically ribbed shells to which the name ("*corbus*" for basket) refers. Among them the limnic *Corbicula* Megerle von Mühlfeld, 1811, which occurs natively in the Middle East, Africa and Madagascar throughout Asia to Australia, holds hyper-invasive neozoon in the New World as well as Europe (Fig. 1), where it was unknown prior to 1924 and the 1970s, respectively. Currently some taxa are rapidly extending their range (e.g., Britton & Morton, 1979; McMahon, 1983; Kinzelbach, 1991, 1992; Ituarte, 1994; Bji de Vaate & Greijdanus-Klaas, 1990; Araujo et al., 1993; Csányi, 1999; Renard et al., 2000; Siripattrawan et al., 2000; Beasley et al., 2003; Beran, 2006), rendering some species of the genus of special ecological importance. In particular, the bicontinental spread of clonal lineages in North and South America is currently viewed as "a portent of things to come" (Lee et al., 2005).

In addition to their extraordinary invasive potential, "Asian clams" are also interesting for their only recently discovered wide spectrum of reproductive strategies. This developmental plasticity involves sexually reproducing species, either gonochoristic or hermaphroditic, and a variety of reproductive features, such as oviparity (e.g., in *Polymesoda, Batissa, Corbicula japonica, C. sandai)*, ovoviviparity (in the majority of *Corbicula* species, such as *C. fluminalis* and *C. fluminea*),

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[1] We dedicate this article to the memory of the Ukrainian malacologist Dr. Alexei Korniushin (1962–2004) in credit for his contributions to the systematics of limnic bivalves.

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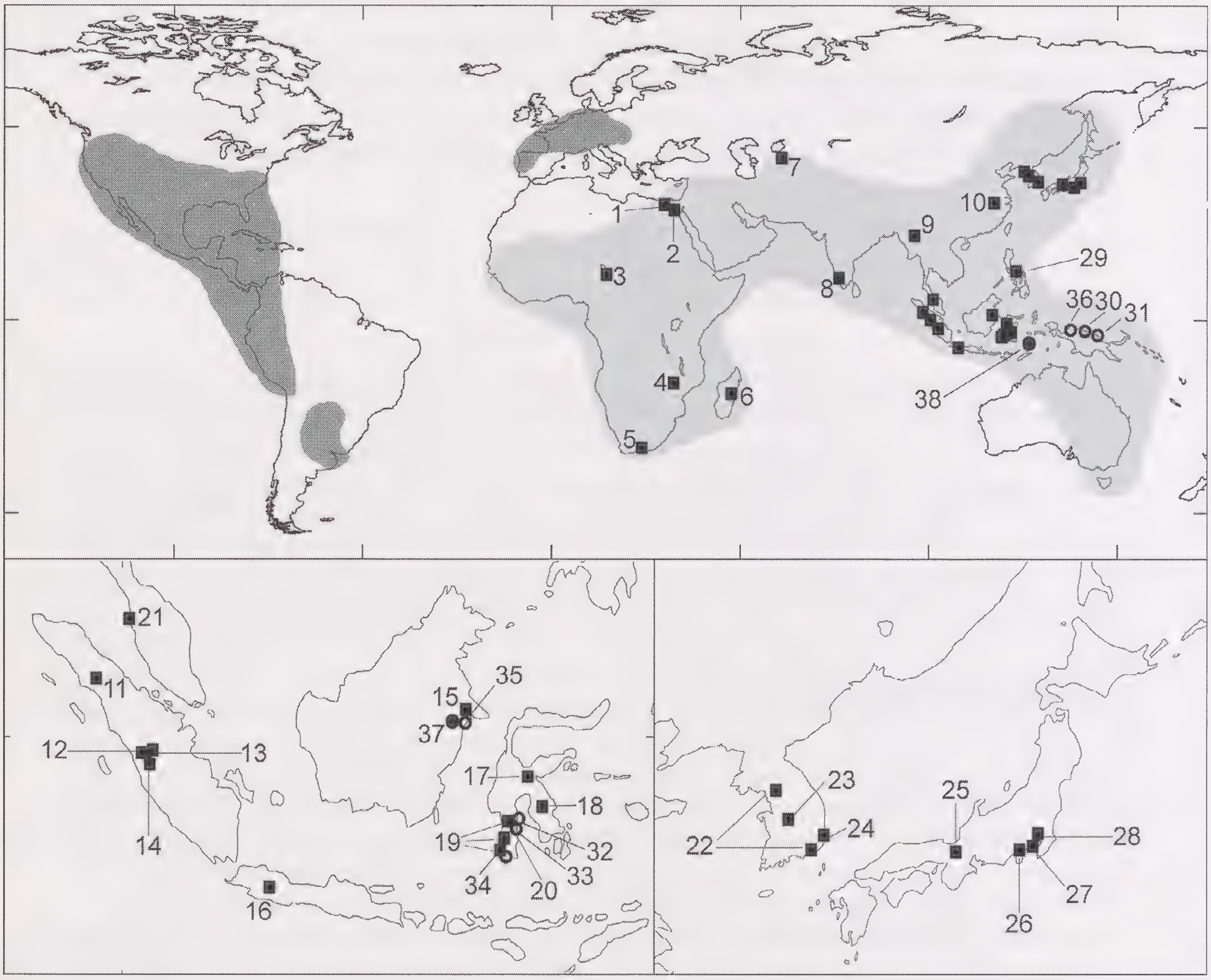

FIG. 1. Native (grey) and non-indigenous distribution (dark grey) of *Corbicula* in the Old World and New World, with type localities of ZMB type material of *Corbicula* taxa (closed squares), *Batissa* taxa (open circles), and "*Cyrena*" taxa (closed circle) (after Morton 1986, additional data from: Beasley et al., 2003; Beran, 2006; Csányi, 1999; Kinzelbach, 1991, 1992; Lee et al., 2005). Indo-Malaysian Archipelago and Eastern Asia enlarged below. 1 – *C. jickeli*, 2 – *C. zelebori*, 3 – *C. tsadiana*, 4 – *C. astartina*, 5 – *C. africana* var. *olivacea*, 6 – *C. sikorae*, 7 – *C. oxiana*, 8 – *C. cochinensis*, 9 – *C. noetlingi*, 10 – *C. largillierti*, 11 – *C. tobae*, 12 – *C. lacustris*, 13 – *C. gustaviana*, 14 – *C. angulifera, C. gibba* and *C. trapezoidea*, 15 – *C. bitruncata*, 16 – *C. javanica*, 17 – *C. possoensis*, 18 – *C. matannensis*, 19 – *C. celebensis*, 20 – *C. subplanata*, 21 – *C. regia*, 22 – *C. elatior*, 23 – *C. producta*, 24 – *C. colorata*, 25 – *C. sandai*, 26 – *C. straminea*, 27 – *C. transversa*, 28 – *C. biformis*, 29 – *C. sayana* and *C. crosseana* (Philippines, without more precise locality), 30 - *Batissa angulata*, 31 – *B. finschi*, 32 – *B. violacea* var. *celebensis,* 33 – *B. violacea* var. *discoidea*, *B. violacea* var. *extensa*, 34 – *B. violacea* var. *macassarica,* 35 – *B. schmidti*, 36 – *B. subtrigona*, 37 – "*Cyrena*" *prona,* 38 – "*Cyrena*" *moluccensis*. Not shown due to imprecise type locality data: *Corbicula japonica* (Japan), *Corbicula oblonga* (presumably Australia), and *Polymesoda cordata* (Mexico).

as well as euviviparity (in *Neocorbicula, C. madagascariensis* and *C. linduensis*); see, for example Ituarte (1994), Byrne et al. (2000), Glaubrecht et al. (2003), Korniushin & Glaubrecht (2003), and Glaubrecht et al. (2006). Another peculiar feature is the genetic structure in *Corbicula*, comprising phenomena such as polyploidy, androgenesis, and clonality, with mechanistically diverse genetic interactions among clones (e.g., Komaru et al., 1997, 1998; Komaru & Konishi, 1999; Qiu et al., 2001; Pfenninger et al., 2002; Park & Kim, 2003; Glaubrecht et al., 2003; Lee et al., 2005).

Although these particular properties may potentially obscure phylogenetic relationships of some (i.e., the clonal) freshwater lineages, it nevertheless renders *Corbicula* an ideal model group for studying evolutionary transformations of reproductive features as proposed by Glaubrecht et al. (2003, 2006) and Korniushin

& Glaubrecht (2003). This holds true in particular with respect to the correlation of freshwater colonization and ctenidial brooding of internally fertilized eggs, embryos, and shelled larvae in specialized brood pouches (marsupia). While hitherto only the South American *Neocorbicula* Fischer, 1887, was known to be euviviparous (Ituarte, 1994), recent studies of taxa from the Indonesian island of Sulawesi (Korniushin & Glaubrecht, 2003; Glaubrecht et al., 2003) and of *C. madagascariensis* Smith, 1882, from Madagascar (Glaubrecht et al., 2006) uncovered new reproductive modes in *Corbicula.* Based on a molecular phylogeny (Glaubrecht et al., 2006), these findings suggest that euviviparity, including matrotrophy, developed independently in different corbiculid lineages.

On the other hand, the systematics of corbiculids is not resolved satisfactorily to date, partly due to the fact that morphological characters alone proved to be equivocal. The state of corbiculid taxonomy may be taken as another prime example for the general controversy in the delineation of species; for a review of this unresolved "species problem" in malacology, see, for instance, Glaubrecht (2004). For example, forms from the African continent are subsumed by some authors under two species-level taxa only, that is, *C. fluminalis* (Müller, 1774) and *C. astartina* Martens, 1860, comprising particularly in the case of the former a plethora of subspecific differentiations (e.g., vars. *africana, olivacea, consobrina*) (Mandahl-Barth, 1988; Korniushin, 2004). Evidently, this conflict also exists for Asian corbiculids, for which "splitters" distinguish several taxa, with Prashad (1924, 1929, 1930) listing up to 70 species, and Brandt (1974) listing 26 species for Thailand alone; for Indonesia, see Djajasamita (1975, 1977). In contrast, "lumpers" accept only two conchologically highly variable, polymorphic taxa (e.g., Morton 1979, 1986). Recently, Korniushin (2004) discussed some of the problems in distinguishing species in Asian and African corbiculids assigned to *C. fluminalis* and *C. fluminea* (Müller, 1774), respectively. This confusion on species identities and taxonomy in corbiculids becomes evident from the comparison of Mandahl-Barth (1988) and Daget (1998) for Africa, Morton (1986) and Harada & Nishino (1995) for Japan, Brandt (1974) and Woodruff et al. (1993) for Thailand, or Djajasasmita (1975, 1977) and Glaubrecht et al. (2003) for Indonesian and particularly Sulawesi taxa; for the particular case of the cementing (i.e., sessile) corbiculid named "*Posostrea*" from Lake Poso on Sulawesi compare Bogan & Bouchet (1998) to Rintelen & Glaubrecht (2006). Accordingly, the confusing taxonomy and unresolved systematics of *Corbicula* today still hampers both fundamental and applied studies in these important limnic bivalves, while such other corbiculid taxa as *Batissa* Gray, 1853, and *Polymesoda* Rafinesque, 1820, are hardly studied at all.

Most recently, several analyses based on mitochondrial sequence data tried to resolve the phylogenetic relationships of corbiculids. Most of these studies found the South American *Neocorbicula* in a sister-group relationship to *Corbicula*, and within this genus the brackish-water *C. japonica* Prime, 1864, from Korea plus *C.* cf. *fluminalis* from China in a basal position. While the freshwater *C. madagascariensis* represents the next basal split, all other Asian, Australian, European, and American morphotypes of *Corbicula* form a cluster with poorly resolved relationships, with morphological and molecular characters sometimes in contradiction (e.g., Siripattrawan et al., 2000; Pfenninger et al., 2002; Glaubrecht et al., 2003; Park & Kim, 2003; Lee et al., 2005; Rintelen & Glaubrecht, 2006; Glaubrecht et al., 2006).

Pilsbry & Bequaert (1927: 339) noted that "it would require intimate acquaintance with the types and long sets of each variety to unravel the extraordinary tangle into which the ... species of *Corbicula* have been woven". Following this sage remark, which can well be read as the credo of biosystematics in general, we here document the extensive corbiculid type material in the Mollusca Collection of the Natural History Museum of the Humboldt University in Berlin (ZMB), being a first step in the course of revisionary taxonomic work for Corbiculidae. We here document types that were ignored in the most recent nomenclatural and type catalogues of *Corbicula* (Counts, 1991; Daget, 1998), as it was the case with other material from the ZMB, which had – albeit being one of the major natural history collections worldwide – long being neglected by systematics (see for other molluscs groups, e.g., Glaubrecht & Salcedo-Vargas, 2000; Korniushin & Glaubrecht, 2001; Köhler & Glaubrecht, 2002, 2006; Scholz & Glaubrecht, 2004; Glaubrecht et al., 2005).

Therefore, the present compilation is intended to contribute to a more stable taxonomy of the family by clarifying and presenting crucial information on the extant types of the ZMB, which is one of the major repositories for primary types of Corbiculidae, in particular for Old World taxa. Given the recent interest in these invasive clams, the focus in this account lies with the freshwater and brackish-water *Corbicula*; it is supplemented by taxa traditionally attributed to *Batissa*, one to *Polymesoda*, and some additional taxa of currently unresolved generic affinity.

MATERIALS AND METHODS

In the absence of a modern systematic revision, no comprehensive account of constituent genera of the Corbiculidae is yet available. A preliminary review, however, allows grouping 13 named generic-level taxa by their geographical distribution. In addition to *Corbicula,* the following, partly obscure, generic names have been applied: (i) for the Neotropics *Cyanocyclas* Blainville, 1818*; Egetaria* Mörch, 1861; *Neocorbicula* Fischer, 1887; *Polymesoda* Rafinesque, 1820; and *Pseudocyrena* Bourguignat, 1854; (ii) for the Ethiopis *Soleilletia* Bourguignat, 1885 (but given erroneously for subfossil marine mesodesmatid bivalves; see Mandahl-Barth, 1988: 112); (iii) for the Oriental *Batissa* Gray, 1853*; Cyrenobatissa* Suzuki & Oyama, 1943; *Cyrenodonax* Dall, 1903 (endemic to Taiwan); *Geloina* Gray, 1840; and *Villorita* Gray, in Griffith & Pidgeon, 1834; as well as *Posostrea* Bogan & Bouchet, 1998, for an endemic corbiculid in Lake Poso on Sulawesi (Rintelen & Glaubrecht, 2006); (iv) for the Australian *Corbiculina* Dall, 1903. The generic name *Cyrena* Lamarck, 1818, was applied by earlier authors (e.g., Philippi 1846–1850; Krauss, 1848; Martens 1897) mostly to some but not all African taxa of *Corbicula*, but is regarded as junior synonym in Pilsbry & Bequaert (1927); nevertheless, the latter still used the family-group name Cyrenidae instead of Corbiculidae.

The ZMB type material mainly covers corbiculids from the Old World and stems in particular from the former curator Eduard von Martens (1831–1904) who himself collected in Southeast Asia in the 1860s. Later, additional material came from other travelling naturalists, for example collections done by Dr. Martin Schmidt on Borneo (Martens 1903b), and by Dr. Otto Finsch on New Guinea, as well as several others travelling to then largely isolated Japan, as reported on by Reinhardt (1877, 1878, 1886).

A total of 45 corbiculid types were found among the approximately 550 lots of the dry collection catalogued in the ZMB, with 33 of *Corbicula*, 8 of *Batissa,* one of *Polymesoda* and three of unresolved generic affinity. Reflecting more than 7% of the 609 nominal taxa listed in Counts (1991) catalogue of fossil and Recent corbiculids, the present account adds considerably to our knowledge; note that the majority of the ZMB types lack in Counts (1991) despite his statement (p. 4) that "every attempt has been made to identify the location of type material for these bivalves".

A total of 28 lectotype designations (for 20 type specimens in *Corbicula*, for six in *Batissa*, and for two in "?*Cyrena*") are made here in accordance with the purpose of Article 74.7.3 of the fourth issue of the *International Code of Zoological Nomenclature* (ICZN, 1999), that is, in order to ensure the name's proper and consistent application. We agree that such designations are best done in the framework of a revision taking into consideration all available type material holdings. However, in those cases in which we have seen problems with the identity of name-bearing type specimens, we have indicated for the stability of taxonomic usage those specimens to be designated, and have given indication as to additional types in other museum holdings. We consider this a valuable step done not as pure curatorial practice, but as leading toward the attempted revision. Again, such a revision in a modern sense is a major task, because it should also comprise, in case of corbiculids, such aspects as genetical variability and clonality that would allow a judgement, for example, on the morphotype/haplotype descrepancy and the problem of clonal lineages of the corbiculid invaders. We here hope to raise interest towards this and to provide information on further type material holdings.

The type localities, as compiled in Figure 1, are given here as in the original description and the original label (in quotation marks, including original crossed-out words and asterisks), respectively, and are compared to contemporary and recent atlases (e.g., *Andrees Allgemeiner Handatlas*, 4th edition, 1900); *The Times Atlas of the World*, 1990) as well as modern geographical reference systems, that is, using the FuzzyG online database (names there are in accordance with the European Commission Joint Research Centre Digital Atlas, ISFERA); the geographical coordinates of the localities were traced with FuzzyG (v2.1), and are given in parentheses.

We like to point out, however, that in cases with imprecise original information, this may result in an unrealistic degree of accuracy. For example, for a location such as "Yokohama", where *Corbicula* was once collected, we give coordinates only in a rough (to the minute) format due to lack of knowledge as to where exactly in Yokohama the types were collected.

Supplementing the corbiculid type material of the ZMB, this collection now holds recent additions (approximately 150 lots) comprising 70–96% ethanol-fixed material collected during field work in Thailand, Indonesia, and Australia by Frank Köhler, Thomas von Rintelen, and Matthias Glaubrecht. This forms the basis on which several phylogenetic and evolutionary studies were based (Glaubrecht et al., 2003; Korniushin & Glaubrecht, 2004; Rintelen & Glaubrecht, 2005; Glaubrecht et al., 2006).

Museum Codens:

BMNH	The Natural History Museum (formerly British Museum, Natural History), London, U.K.
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, U.S.A.
MHNB	Museum d'Histoire Naturelle, Basel, Switzerland
SMF	Senckenberg Naturmuseum und Forschungsinstitut, Frankfurt am Main, Germany
ZMA	Zoölogisch Museum, Amsterdam, The Netherlands
ZMB	Museum für Naturkunde, Humboldt University, Berlin, Germany (formerly Zoological Museum Berlin)
ZMZ	Zoologisches Museum, Universität Zürich, Switzerland

ALPHABETICAL LIST OF CORBICULID TYPE MATERIAL IN THE MALACOZOOLOGICAL COLLECTION OF THE BERLIN MUSEUM OF NATURAL HISTORY (ZMB)

Corbicula Megerle von Mühlfeld, 1811

africana Krauss, 1848

Corbicula africana Krauss, 1848: 8, pl. 1, fig. 8.

Comments: For taxonomic remarks, including type localities and the status of the types, see also under *olivacea* Krauss, 1848. The only type specimen to which the name *C. africana* unequivocally is related is the specimen figured in the original description. All other specimens of the original series of Krauss, however, refer to one of the two variety-names *olivacea* and *albida*. The whereabouts of the holotype is unclear, and it is not possible to identify this specimen among the original series. This causes serious taxonomical problems as to the proper use of the name *C. africana*.

Mandahl-Barth (1988), Daget (1998), and most recently Korniushin (2004) considered *C. africana* to be a valid species. However, they did not recognize the reasoning of Krauss (1848), who stated that the varieties of *C. africana* that he introduced are identical with *C. pusilla* and *C. radiata*, respectively (for details see under *olivacea*). The latter two taxa, however, are considered as synonyms of *C. fluminalis consobrina* (Cailliaud, 1827) by Mandahl-Barth (1988) and Daget (1998). Because of these difficulties, it is not possible to judge on the status of *C. africana* as long as the holotype has not been identified. This case perfectly demonstrates that the African *Corbicula* taxa are in desperate need of a modern systematic revision (see Introduction).

angulifera Martens, 1897

Corbicula angulifera Martens, 1897: 116, pl. 7, figs. 28–31.

Type Locality: "Sumatra: Danau di atas und Danau di bawah"; Indonesia, Sumatra: Lake Diatas (01°05'S, 100°45'E) and Lake Dibawah, near Solok (01°05'S, 100°45'E).

Type Material: Syntypes ZMB 170.005 (6 articulated pairs of valves, 1 left valve, 1 right valve; Fig. 2A); with labels indicating locus typicus and "leg. Weber" (see Counts, 1991: 8).

Comments: Because there are two labels found in the single lot extant, we assume that material from the two type localities has been lumped. Further type material is likely housed with the ZMA, because Martens description was based on material collected by Max Weber during his expedition, and most of this material is housed with the ZMA (Djajasasmita, 1977). In the ZMB, there are only parts of the original series examined by Martens. Both Djajasasmita (1977) and Prashad (1930) treat *C. angulifera* as synonym of *C. sumatrana*.

astartina Martens, 1860

Cyrena astartina Martens, 1860: 219–220, pl. 3, figs. 6–7.

Type Locality: "Tette im Zambezefluss"; Mozambique: Zambezi River, Tete (16°09'14"S, 33°35'09"E).

Type Material: Lectotype, **here designated**, ZMB 4.655a (pair of valves, of which a single valve was originally figured; "*Cyrena astartina* Martens, Tette, Peters"; Fig. 2B–C); paralectotypes ZMB 4.655b (7 pairs of valves); paralectotypes BMNH 1902.5.26.8 (not seen; mentioned by Counts 1991: 9).

Comments: Volume 6 of the German journal *Malakozoologische Blätter*, originally intended for 1859 (as also printed e.g. on plate 3 of this volume) was not published before 1860, as can be judged from the pagination of the front page (see also Mandahl-Barth, 1988; Kabat & Boss, 1997).

Counts (1991: 9) gives "Tette in the Zambezi River, Lorenzo Marques, Africa" as type locality, which is erroneous, because Lorenzo Marques is the Portuguese name of Maputo far from Tete. However, the specimen in the BMNH was donated by Martens only with the locality data "Tette" (Jonathan Ablett, BMNH, pers. comm.).

Based on shell morphology, recent authors (Daget, 1998; Mandahl-Barth, 1988) have considered *C. astartina* as a valid species; neither reproductive biology nor molecular genetics are known to date for comparative purpose, for example, with *C. fluminalis*, as recently discussed by Korniushin (2004).

biformis Reinhardt, 1877

Corbicula biformis Reinhardt, 1877: 70; 1878: 189, pl. 5, fig. 3.

Type Locality: Not given by Reinhardt (1877); here restricted to Nikko (Honshu, Japan) (36°44'53"N, 139°36'47"E); see also under Comments.

Type Material: Holotype SMF 6010 ("type, Japan, Nikko-Gebirge, ex. Dönitz"); shell measurements: 16.8 x 15.4 mm (R. Janssen, SMF, pers. comm.).

Comments: We consider the specimen SMF 6010 as the holotype. As we reconstruct from available evidence, this specimen was collected by Dönitz in Nikko, about 125 km N of Tokyo, and fits very well to the measurements given by Reinhardt (1877: 70). Later, this specimen is listed by the same author (Reinhardt, 1878: 190) with these parameters as collected by Dönitz. The same information is found on

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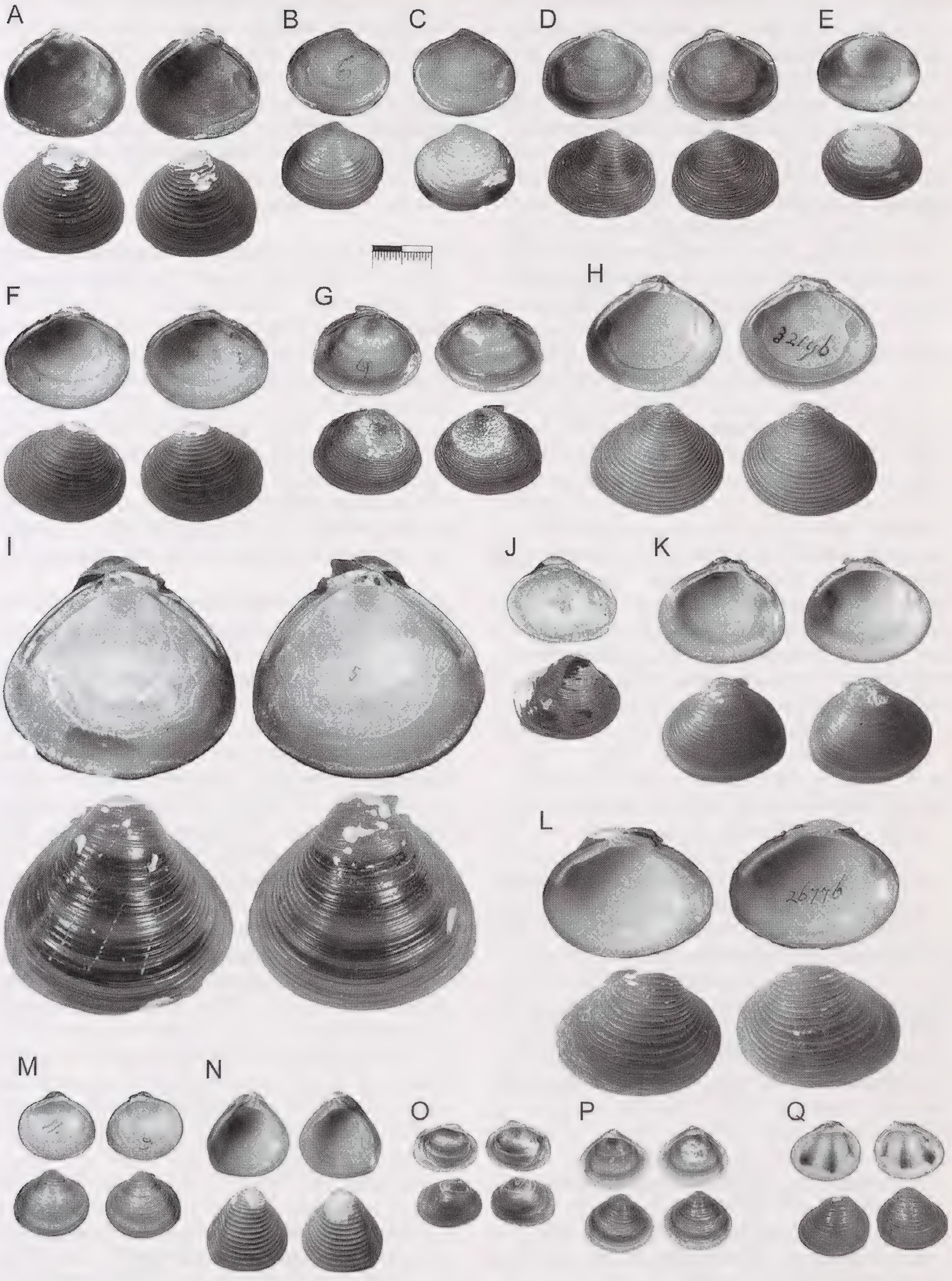
FIG. 2. Type Material from ZMB collection of *Corbicula* (natural size). A: Syntype of *Corbicula angulifera* Martens, 1897 (ZMB 170.005; Indonesia, Sumatra: Lake Diatas and Lake Dibawah, near Solok); B: Lectotype of *Cyrena astartina* Martens, 1860 (ZMB 4.655a; left valve depicted in Martens (1860: pl. 3, fig. 6); Mozambique: Zambezi River, Tete); C: Lectotype of *Cyrena astartina* Martens, 1860 (ZMB 4.655b; right valve, same individual but not depicted in the original description); D: Lectotype of *Corbicula bitruncata* Martens, 1908 (ZMB 108.826a; Indonesia, Kalimantan Timur, Guleh River, W of Sangkulirang Bay); E: Lectotype of *Corbicula celebensis* Martens, 1897 (ZMB 103.018; left valve; Indonesia, Sulawesi: Macassar); F: Paralectotype of *Corbicula celebensis* Martens, 1897 (ZMB 103.021; Indonesia, Sulawesi: lake near Tempe); G: Lectotype of *Corbicula papyracea* Heude var. *colorata* Martens, 1905 (ZMB 55.628; South Korea: Prov. Kyongsang-namdo, Ulsan); H: Syntype of *Corbicula crosseana* Prime, 1864 (ZMB 32.196; Philippines); I: Lectotype of *Corbicula elatior* Martens, 1905 (ZMB 55.624; South Korea: Prov. Kyongsang-namdo: Nak-tong River); J: Syntype of *Corbicula gibba* Martens, 1897(ZMB 170.003; left valve; Indonesia, Sumatra: Lake Diatas); K: Syntype of *Corbicula japonica* Prime, 1864 (ZMB 32.198; Japan); L: Paralectotype of *Cyrena orientalis* Lam. var. *javanica* Mousson, 1849 (ZMB 26.776; Indonesia: Java, Tjikoja); M: Lectotype of *Corbicula jickeli* Clessin, 1878 (ZMB 37.444a; Egypt: near Cairo, in canal); N: Lectotype of *Corbicula lacustris* Martens, 1897 (ZMB 170.001a; Indonesia, Sumatra, Lake Singkarak, in depth of down to -50 m); O: Lectotype of *Corbicula oblonga* Clessin, 1879 (ZMB 170.064a; Australia?); P: Syntype of *Cyrena africana* var. *olivacea* Krauss, 1848 (ZMB 4.850; South Africa: Swellendam, Gauritz River); Q: Lectotype of *Corbicula regia* Clessin, 1878 (ZMB 170.404a; Malaysia, Penang). Scale bar = 10 mm.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the label associated with the SMF 6010 holotype. Counts (1991: 10) listed the holotype (SMF 6010) and a paratype (SMF 6014) originating from "Tokyo, Japan". However, since the description is clearly based on only a single specimen, SMF 6014 has no type status.

In his description, Reinhardt (1877: 70) did not give the type locality. Further species described in the same article originated from different provenances, such as "Yeddo" = Tokyo (leg. Dönitz), Tokyo, Kanga-Yashki (leg. Hilgendorf), and Tokyo, Uweno (leg. Hilgendorf). In a later report, Reinhardt (1878: 189–191) mentioned that the type specimen is a juvenile, and he published a more comprehensive description along with a figure that is based on further material collected by Martens in Yokohama and by Johannes Justus Rein (1835–1918) also in Japan during his travels by order of the Prussian government in the years 1873–1875 (Hohmann, 1982). Material from these collectors is also extant in the SMF (no. 6012, 6013, 6014, leg. Rein and Martens) (R. Janssen, pers. comm.). This material, however, was not available to Reinhardt when he described the taxon in 1877, and therefore does not qualify as types.

Prashad (1924) considered *C. biformis* as synonym of *C. japonica*.

bitruncata Martens, 1908

Corbicula bitruncata Martens, in Martens & Thiele, 1908: 290–291, pl. 6, fig. 25.

Type Locality: "Sungei Goleh, im Westen der Sangkulirang-Bai"; Indonesia, Kalimantan Timur, Guleh River, W of Sangkulirang Bay (1°11'08"N, 117°51'44"E).

Type Material: Lectotype, **here designated**, ZMB 108.826a (pair of valves; "*Corbicula bitruncata*, Sungei Goleh, W. d. Sang Kulirangbai, M. Schmidt leg. 1902"; Fig. 2D); paralectotypes ZMB 108.826b (4 pairs of valves).

Comments: The specimen that is most likely depicted in the original description, as can be judged by comparison of the shell with the enlarged figure, is designated as the lectotype of *C. bitruncata*.

celebensis Martens, 1897

Corbicula celebensis Martens, 1897: 113–114, pl. 7, figs. 11–13.

Type Locality: "Celebes: bei Makassar von mir, im Fluss bei Maros, im See bei Tete-adji, in dem von Tempe und im Fluss Minralang bei Tempe von Prof. Weber gefunden"; Indonesia, Sulawesi: herein restricted to Makassar (5°09'S, 119°26'E) (leg. Martens). Localities of paralectotypes: River near Maros, lake near Teteadji, lake near Tempe, Walanae River near Tempe (leg. Weber).

Type Material: Lectotype, **here designated**, ZMB 103.018 (left valve, "*Corbicula celebensis*, Makassar, v. Martens, Taf. VII fig. 11–13"; Fig. 2E); paralectotypes ZMB 103.019 (6 pairs of valves, "*Corbicula celebensis* m., Maros Fluss, Celebes, Weber"); paralectotypes ZMB 103.020 (11 pairs of valves, 47 valves, "*C. celebensis*, Tete adji, See bei Tete adji Celebes, Weber"); paralectotype ZMB 103.021 (6 pairs of valves, 1 left valve, 1 right valve, "*C. celebensis* Marts, See v. Tempe, Celebes, Weber"; Fig. 2F); ZMB 103.022 (pair of valves, "*C. celebensis*, Fluss Minralang, Celebes").

Comments: Here we designate the specimen ZMB 103.018, from material of Weber's collection that was described by Martens (1897), as lectotype of *C. celebensis*. The single valve figured by Martens (1897: pl. 7, fig. 11) is recognisable by its particular damage of periostracum and exactly coincides with the dimensions provided (Martens 1897: 114): L = 17.5 mm, H = 14 mm, W = 9 mm). Other figured specimens from the same locality, that is, Makassar (Martens, 1897: pl. 7, figs. 12–13), were not found in the ZMB collection. Djajasasmita (1977) noted for material in the ZMA the locations "River Minralang near Tempe, 1889, M. Weber coll. (ZMA)", however without indicating that this possibly also represent type material. Prashad (1930) published photos from a potential type lot, however without stating clearly which material it was and where it is. In his introduction, though, he mentioned that he used ZMA material. Counts (1991: 12) was not able to trace type material of this taxon.

The type material of *C. celebensis* from four of the five sites (Teteadji, Maros, Makassar, Tempe) resembles *C. javanica*, in being oval, symmetric or posteriorly elongated, widely and coarsely ribbed; this is also evident in the *C. subplanata* type material which is also from Minraleng. In contrast, the type material from Minraleng looks different, resembling "*C. fluviatilis*", with denser ribbing, and is not oval-elongate.

Prashad (1930) treated *C. celebensis* as a separate species. However, Djajasasmita (1975, 1977) synonymized this taxon with Martens' *C. subplanata* without giving explicit reasoning for this. We here follow his decision regarding him as being first revising author; see under *C. subplanata*.

colorata Martens, 1905

Corbicula papyracea Heude *var. colorata* Martens, 1905: 67, pl. 2, fig. 10.

Type Locality: "Ulsan, nördlich von Tongdosa, Provinz Kyöngsangdo, Korea"; South Korea: Ulsan-gwangyoksi, Kyongsang-namdo (35°32'N, 129°18'E).

Type Material: Lectotype, **here designated**, ZMB 55.628 (pair of valves; "*Corbicula papyracea colorata,* Ulsan"; Fig. 2G); paralectotypes ZMB 38.438 (8 pairs of valves; "*Corbicula papyracea,* Ulsan, leg. Gottsche").

Comments: Martens (1905: 67) mentioned a series of nine specimens collected by Gottsche, on which his description is based. ZMB 55.628 contains the specimen figured in the original description, as can be judged from a comparison of the shell with the figure. This specimen herein is designated as the lectotype of *C. papyracea var. colorata*. ZMB 38.438 contains the residual 8 specimens mentioned by Martens. The material is not labelled as var. *colorata*, but there is no doubt that these indeed are types of this variety, because Martens (1905) reported *C. papyracea* from Sangju, but not from Ulsan. Counts (1991: 28) was not able to trace type material.

Prashad (1930) treated *C. colorata* as a synonym of *C. nitens.*

crosseana Prime, 1864

Corbicula crosseana Prime, 1864: 72, fig. 10.

Type Locality: "Insulae Philippinenses, collected by Wheatley and Prime"; Philippines.

Type Material: Syntype ZMB 32.196 (pair of valves; "*Corbicula crosseana* Prime, Philippinen, Prime ex coll. Gundlach"; Fig. 2H).

Comments: According to Johnson (1959: 449) and Counts (1991: 14), the holotype is in the MCZ (MCZ 135635). However, Prime did not explicitly designate a holotype and his description apparently is based on more than a single specimen. The mentioning of a holotype by Johnson (1959) and Counts (1991) probably reflects that only a single specimen was available to them. For that reason, we consider the specimen in the MCZ rather a syntype. The specimen in the ZMB does not represent the shell depicted by Prime, as can be concluded from a comparison with the figure. The material was obtained by the ZMB from Gundlach along with a note that it originated from Prime. We therefore consider it as being part of a syntype series that was subsequently dispersed by Prime.

According to Prashad (1930), *C. crosseana* is a synonym of *C. squalida.*

elatior Martens, 1905

Corbicula elatior Martens, 1905: 65–66, pl. 2, figs. 5–6.

Type Locality: "Mittlerer Arm des Naktonggang, Hauptfluss der Provinz Kyöngsangdo, zwischen Tongnai und Kimhai. Imjingang bei Imjin, Provinz Kyöngkwido, Korea"; South Korea: herein restricted to Naktong River near Kimhae, Kyongsang-namdo (35°11'57"N, 128°54'00"E). Locality of paralectotypes: Prov. Kyonggi-do: Imjin River near Imjin.

Type Material: Lectotype, **here designated**, ZMB 55.624 (1 pair of valves; "*Corbicula elatior** Marts., Naktonggang"; Fig. 2I); paralectotypes ZMB 38.439 (10 pairs of valves, 1 right valve; "*Corbicula elatior* jung, Naktonggang, mittl. Arm Korea, Gottsche"); paralectotypes ZMB 38.429 (6 pairs of valves, 1 right valve; "*Corbicula* No. 1, N'aktonggang, Gottsche"); paralectotypes ZMB 38.430b (3 pairs of valves, 1 tiny right valve; "*Corbicula* no. 2, Imjingang bei Imjin 20/9 Gottsche"); paralectotype ZMB 55.626 (pair of valves; specimen depicted in fig. 6 as "*C. elatior*?").

Additional Material: Material of unclear status, ZMB 38430a (5 pairs of valves, 1 left valve; "*C. elatior*, Imjingang bei Imjin, Gottsche").

Comments: The specimen ZMB 55.624 represents the shell depicted in Martens' fig. 5 and therefore is designated as the lectotype. Martens (1905: 65) refered to 6 adult and 12 younger specimens from the Naktonggang (Nak-tong River) and a further four specimens from the Imjing River. The lots ZMB 38.429, 38.439, and 55.624 comprise together valves of 18 specimens, which fits the number given by Martens. Therefore, the lot ZMB 38.429 is also considered of type status, although the label does only give "*Corbicula* No. 1". The lot ZMB 38.430b apparently contains valves of the four specimens mentioned by Martens from the Imjingang. Although the label gives only "*Corbicula* No. 2", this is considered as having type status. The status of the lot ZMB 38.430a, which originates from the same series, remains unclear; apparently, these shells were not considered conspecific with the specimens of ZMB 38.430b by Martens because they were slightly different. Therefore, this lot likely contains no types. The lot ZMB 55.626 contains the specimen depicted in Martens' fig. 6, which was only tentatively assigned to *C. elatior* by Martens (1905: 66). Counts (1991: 16) was not able to trace type material.

Prashad (1930) treated *C. elatior* as synonym of *C. fluminea.*

gibba Martens, 1897

Corbicula gibba Martens, 1897: 121, pl. 7, figs. 25–27.

Type Locality: "Sumatra: im See Danau di atas"; Indonesia, Sumatra, Lake Diatas (01°05'S, 100°45'E).

Type Material: Syntypes ZMB 170.003 (left valve; "*Corbicula gibba* Marts* Danau di atas Sumatra"; Fig. 2J); syntypes ZMB 170.004 (3 pairs of valves, 3 right valves, 2 left valves; "*Corbicula gibba* n., Danau di atas, Sumatra, Weber").

Comments: Counts (1991: 19) mentioned the ZMB as type repository. Neither of the type specimens housed with the ZMB matches the illustrations in Martens (1897: pl. 7, figs. 25–27) perfectly. Because further type specimens are deposited with the ZMA (Djajasasmita 1977), who refers to material from "Danau Diatas, West Sumatra, 1888, M. Weber coll.", we refrain here from a lectotype designation.

Both Prashad (1930) and Djajasasmita (1977) treated *C. gibba* as synonym of *C. sumatrana.*

gustaviana Martens, 1900

Corbicula gustaviana Martens, 1900: 16.

Type Locality: "Danau Baru"; Indonesia, Sumatra: Lake Baru (approx. 0°19'S, 103°16'E).

Type Material: Lectotype, **here designated**, ZMB 51.797a (pair of valves; "*Corbicula gustaviana* Marts, Danau Baru Fluss Indragiri [= Inderagiri River], O. Sumatra, zahlreiche Exemplare, Gustav Schneider"; Fig. 3A); paralectotypes ZMB 51.797b (3 pairs of valves).

Comments: The largest shell of the type lot is herein designated as the lectotype. Counts (1991: 20) did not mention the ZMB as holding types but noted paratypes in the SMF. However, the original series collected by Schneider in Lake Baru on the island of Sumatra, and of which specimens deposited with the ZMB were described by Martens, went through the hands of several collectors. Here we suggest that the SMF material (SMF 168187/3, coll. C. Bosch ex H. Rolle, ex coll. Gustav Schneider) actually represents topotypical material only, since we have no indication that it was in Martens' hands at the time of his species description. The type locality originally given on the label is ambiguous, as it combined a lacustrine location (i.e. Danau (= lake) Baru with a riverine ("Fluss Indragiri").

Djajasasmita (1977) treated *C. gustaviana* as separate species, as did Prashad (1930); the latter, however, also noted that it might be a form of *C. javanica.* This latter taxon shows strong phylogenetic affinity to Korean *C. fluminea*, according to the phylogenetic analyses in Glaubrecht et al. (2003); thus, possibly *C. gustaviana* represents only a local form of *C. fluminea.* Note that this latter taxon is similar to, for example, *C. elatior* from Korea, as well as to some introduced forms in the U.S. (Fehér, pers. observation).

japonica Prime, 1864

Corbicula japonica Prime, 1864: 68–69, fig. 15.

Type Locality: "Japonia (fide Gulick) collect. Wheatley, Jay, Browne, Cooper, Lyc. Hist. Nat., Prime and Lea"; Japan.

Type Material: Syntypes ZMB 32.198 (2 pairs of valves ex coll. Gundlach; "*Corbicula japonica* Prime, Japan, Gulick Prime"; Fig. 2K); syntypes ZMB 170.411 (2 pairs of valves ex coll. Dunker; "*Corbula japonica*, von Mr Prime selbst erhalten").

Comments: According to Johnson (1959: 457) and Counts (1991: 22), types (therein indicated as holo- and paratypes) are deposited with the MCZ. However, Prime did not explicitly desginate a holotype, and his description apparently is based on more than one specimen. Today, the MCZ holds two lots: MZC 152.904, 1 spec. with 2 valves, and MCZ 187468, 2 spec. with 4 valves (A. Baldinger, pers. comm, May 2006). For that reason, we consider the specimens of the MCZ as well as those in the ZMB to be syntypes.

javanica Mousson, 1849

Cyrena orientalis Lam. var. *javanica* Mousson, 1849: 86, pl. 15, fig. 2.

Type Locality: "Tikojia"; Indonesia: Java, Tjikoja (7°06'S, 107°30'E).

Type Material: A lectotype has been designated from ZMZ material (ZMZ 532.199, with paralectotypes ZMZ 532.199a, 532.200); see Glaubrecht et al. (2003). Additional paralectotypes: ZMB 26.776 (2 pairs of valves; ded. Mousson "*Corbicula javanica* Mss., Tjikoya, leg. Zollinger Java, Mss"; Fig. 2L).

Comments: Mousson (1849: 86) states that he had more than 60 specimens. There is no doubt that the material in the ZMB is of type status, since it originates from the original series collected by Zollinger and was obtained from Mousson. In the description, the line "Tikojia. Wird vom Volke Remis genannt und gegessen" [meaning of sentence ambigous; it can either be translated with "Tikojia. Is called Remis and is eated by the people" or alternatively "Is called Tikojia and eaten by the Remis people"]. In an online reference on the life and collecting activities of Heinrich Zollinger (National Herbarium of the Netherlands, looked up at Nov. 2005), information is presented that in 1841 Zollinger travelled to the Tjikoja Estate in the western part of Java (Bantam). Consequently, we believe that "Tikojia", a misspelling of Tjikoja (see also Djajasasmita 1977), refers to the type locality, rending our first translation above more likely. Thus, "Tjikoya" on the label of the ZMB material probably refers to the type locality.

jickeli Clessin, 1878

Corbicula jickeli Clessin, 1878: 163, pl. 29, figs. 1–2.

Type Locality: "Afrika, bei Cairo in einem Nilkanale (Jickeli)"; Egypt: near Cairo, in a canal.

Type Material: Lectotype, **here designated**, ZMB 37.444a (pair of valves; "*Corbicula jickelii* Cless., Cairo, Jickeli"; Fig. 2M); paralectotypes ZMB 37.444b (2 pairs of valves).

Comments: The lectotype is designated herein because it fits well to the enlarged figure in the original description (pl. 29, fig. 1), as well as to the measurements given by Clessin (1878), with shell breadth = 12.6 mm instead of 13. It is not known if further type specimens may exist in other museum collections because Clessin (1878) did not mention where his specimens were deposited. Counts (1991: 22) was not able to trace type material.

Both Daget (1998) and Mandahl-Barth (1988) treated *C. jickeli* as a synonym of *C. consobrina* Cailliaud, 1827.

lacustris Martens, 1897

Corbicula lacustris Martens, 1897: 118–119, pl. 7, figs. 20–24.

Type Locality: "Sumatra: See von Singkarah, bis zu einer Tiefe von 50 Metern"; Indonesia, Sumatra, Lake Singkarak, in depth of down to -50 m (0°36'S, 100°32'E).

Type Material: Lectotype, **here designated**, ZMB 170.001a (1 pair of valves; "*Corbicula lacustris* Marts., See v. Singkarah, Sumatra, Taf. VII, fig. 20–24"; Fig. 2N); paralectotype ZMB 170.001b (1 pair of valves, juvenile as figured in pl. 7, fig. 21; "*Corbicula lacustris* Marts., See v. Singkarah, Sumatra, Taf. VII, fig. 20–24"); paralectotypes ZMB 170.002 (16 pairs of valves, 18 valves; "*Corbicula lacustris* Marts., See v. Singkarah, Sumatra", "See v. Singkarah, 50 Met."); paralectotype ZMB 54.371 (soft body without shell, in alcohol).

Comments: The specimen ZMB 170.001a is designated as the lectotype as it most likely represents the specimen depicted in pl. 7, figs. 22-23. The shell depicted in fig. 20 is not among the specimens in the ZMB. Additional type material may be deposited with the ZMA, among them the specimen depicted in fig. 20. The specimen of the lot ZMB 170.000 from the same series was sorted out by Martens as being distinct. This can be concluded from the label stating "*C. lacustris* var. cf *pullata*, Singkarah". Therefore, these specimens do not qualify as types of *C. lacustris*.

Djajasasmita (1977) treated *C. lacustris* as a synonym of *C. sumatrana* Clessin collected from Singkarah Lake on Sumatra, albeit without given additional hints for this decision. Prashad (1930) treated it as a distinct species, which he considered to be "closely allied and derived from" *C. sumatrana*, since, as he wrote "it differs from *C. sumatrana* in the outline of the shell, the sculpture being less strongly developed and more irregular and the hinge more strongly arched." Prashad, who probably had not seen the ZMB material, seems to be incorrect, since the sculpture of *C. lacustris* is as strong and as regular as, for example, that of the types of *C. angulifera* (which were studied here as conspecific with *C. sumatrana*; see under that species). In concert with the occurrence of both very similar forms in the same lake on Sumatra, we see to date no strong arguments for considering *C. lacustris* as being distinct.

largillierti Philippi, 1844

Cyrena largillierti Philippi, 1844: 163.

Type Locality: "China, in flumine Yang-tse-kiang; communicavit cl. Largilliert"; China: Yangtsekiang (ex coll. Largilliert).

Type Material: Lectotype, **here designated**, ZMB 170.357a (pair of disarticulated valves; "Yang-tse-kiang", ex coll. Albers, ex coll. Philippi; Fig. 3C); paralectotype ZMB 170.357b (pair of valves).

Comments: We designate the lectotype based on the only type material currently known to exist. This material was given to Friedrich Albers by Rudolph Amandus Philippi (1808–1904). Due to known frequent exchange of material among collectors of this time we anticipate that additional type material might be present in other collections. For example, Counts (1991: 23) mentioned potential syntypes to be held by the MCZ and the USNM originating from Heude's collection. However, the type status of this material is in need of confirmation. We have no information as to whether Philippi ever exchanged material with Heude, which would explain why Heude owned some of Philippi's types. The species was later figured by Philippi (1846: p. 75, pl. 1, fig. 1).

Corbicula largillierti, treated by Prashad (1930) as synonym of *C. fluminea*, was reported to be introduced to the Rio de la Plata catchment in South America, where it occurs syntopically in certain localities with the allochthonous *C. fluminea* (Ituarte 1984). The latter author only reported on conchological evidence for the distinction but did not find anatomical or reproductive biological features to vary. Based on molecular sequence data, Lee et al. (2005) differentiated *C. largillierti* cautiously as "New World form C" from *C. fluminea* as "New World form A". According to their analyses, form C clusters with *Corbicula* haplotypes found in France (Renard et al., 2000), for which the identity as either *C. fluminea*, *C. fluminalis*, or a new cryptic species remained open, but which turned out to be distinct from form A from the same site. Given the clonal nature assumed for many *Corbicula* populations (Lee et al., 2005), the species identity will be very difficult to ascertain. Unfortunately, no "*C. largillierti*" from the type locality has been examined so far.

matannensis P. Sarasin & F. Sarasin, 1898

Corbicula matannensis P. Sarasin & F. Sarasin, 1898: 92, pl. 9, figs. 158–160.

Type Locality: "Matanna-See"; Indonesia, Sulawesi: Lake Matano (2°30'S, 121°20'E).

Type Material: Syntype ZMB 50.799 (pair of valves; "*Corbicula matannensis* SS, S Celebes, Matana See, Sarasin's S.G."; Fig. 3B).

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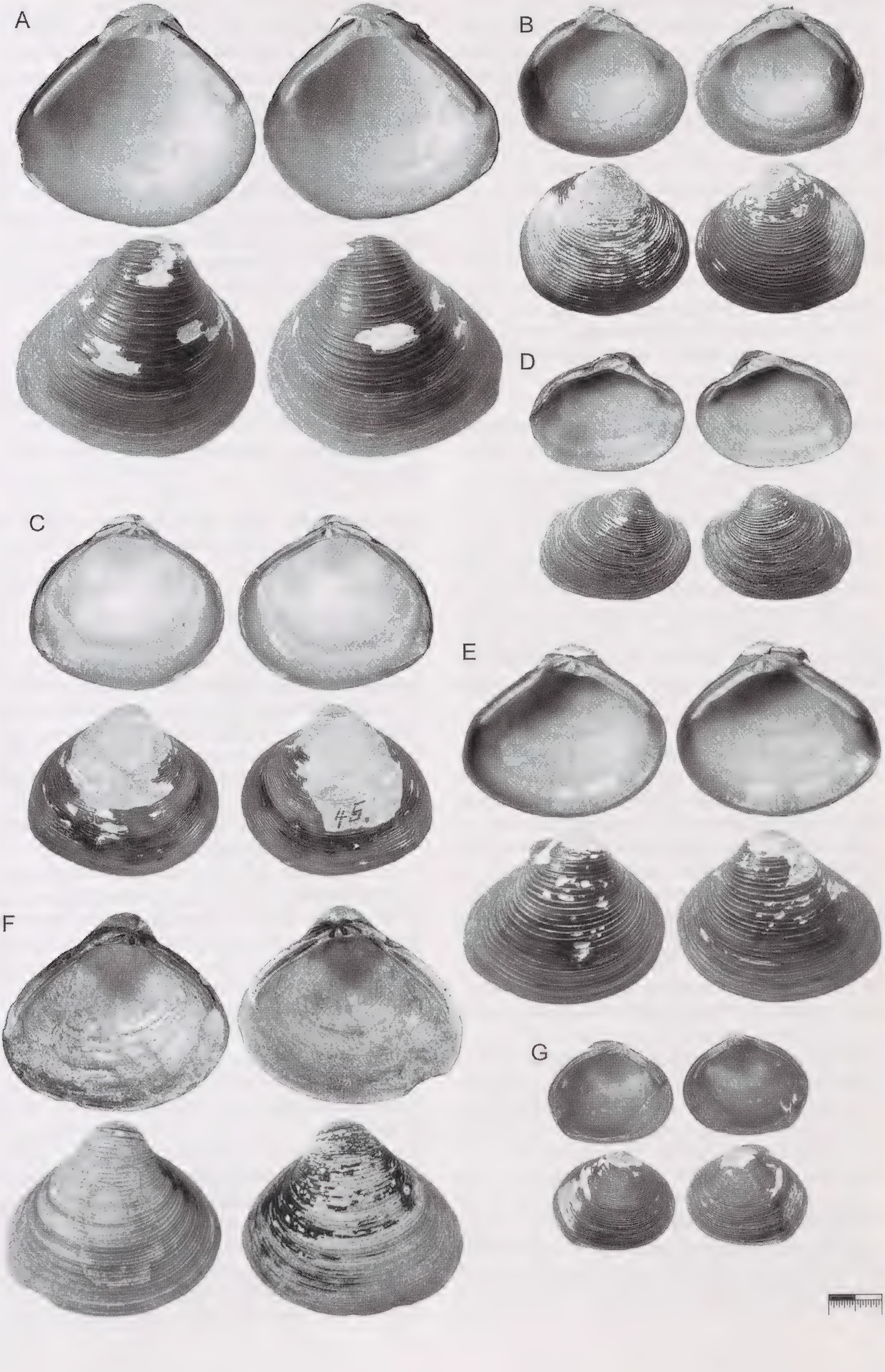
FIG. 3. Type Material from ZMB collection of *Corbicula* (natural size), continued. A: Lectotype of *Corbicula gustaviana* Martens, 1900 (ZMB 51.797a; Indonesia, Sumatra: Lake Baru); B: Syntype of *Corbicula matannensis* P. Sarasin & F. Sarasin, 1898 (ZMB 50.799; Indonesia, Sulawesi: Lake Matano); C: Lectotype of *Cyrena largillierti* Philippi, 1844 (ZMB 170.357a; China: Yangtsekiang); D: Lectotype of *Corbicula noetlingi* Martens, 1899 (ZMB 51.137; Myanmar: North Shan Hpaung); E: Lectotype of *Corbicula producta* Martens, 1905 (ZMB 55.625a; South Korea: Prov. Ch'ungch'ong-namdo, Keum River near Kongju); F: Lectotype of *Cyrena (Corbicula) fluminalis* var. *oxiana* Martens, 1876 (ZMB 27.360b; Uzbekistan: dry river bed of a tributary of the Amu-Darja in the area if Khiva between Sheikh-Dsheili and Kalendar-chana); G: Syntype of *Corbicula possoensis* P. Sarasin & F. Sarasin, 1898 (ZMB 50.798; Indonesia, Sulawesi: Lake Poso). Scale bar = 10 mm.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Comments: The type material has been obtained by the museum from Paul and Fritz Sarasin. Further types are deposited with the museum in Basel (MHNB 43A; non vide), according to Counts (1991: 24). Counts (1991: 24) used a misspelled version of the name ("*matanensis*").

noetlingi Martens, 1899

Corbicula nötlingi Martens, 1899: 47, pl. 4, figs. 7–9.

Type Locality: "In einer kleinen Lache (small pool) bei Hpaung, nördliche Shan-Staaten,... von Bakmio"; Myanmar: North Shan, [perhaps Hpaung-daw] and Bakmio.

Type Material: Lectotype, **here designated**, ZMB 51.137 (pair of valves; "*Corbicula nötlingi* Marts * Hpaung Obw. Birma, Nötling, [unreadable handwriting] Arch. Nat. 1899. 4 8.9!"; Fig. 3D); paralectotypes ZMB 49.669 (28 pairs of valves; "*Corbicula* cf *moussoniana* Dh, small pool near Hpaung Northern Shan states, 1890 Nötling"); paralectotype ZMB 51.138, (pair of valves; "*Corbicula nötlingi* Marts. var., fig 7. Bakmio, Nötling, Arch Nat 1899 4, 7!"); paralectotypes ZMB 49.670 (20 pairs of valves; "*Corbicula moussoniana* Dh, Bakmio Ob Burma, Nötling").

Comments: The specimen ZMB 51.137 likely represents the shell depicted in pl. 4, fig. 8, and is therefore designated as the lectotype of *C. noetlingi*. The specimen ZMB 51.138 represents the shell depicted in pl. 4, fig. 7. Kabat & Boss (1997) mentioned a holotype in the ZMB. However, Martens' description is not based on a single specimen as can be concluded by the statement "Bis 29 mm lang, …." [up to 29 mm long…], which refers to a series of specimens. In the first line of his description, Martens (1899) states "Wahrscheinlich *Moussoniana* (Deshayes) Reeve conch. icon. XX fig. 97, 1878 (nicht *C. moussoni* Desh. catal. Brit. Mus. 1854 p. 227"; which means that he believes that this newly described species may be identical with a species depicted earlier by Reeve (1878). Due to the fact that Martens (1899) based his description on a series of specimens that he considered likely to represent "*C. moussoniana* Reeve, 1878", we consider also the series of specimens of the lot ZMB 49.669 as having type status although being labelled with "*Corbicula* cf *moussoniana*". Furthermore, he mentions "Eine kleinere Varietät, Fig. 7, …, von Bakmio". However, since this variety has not been named by Martens, the according material (ZMB 51.138; ZMB 49.670) is considered as being type material of *C. noetlingi*.

For unknown reasons, Counts (1991: 27) statement that "*notlingi bakmio* Martens … Included in the type series of *Corbicula notlingi*" should be a *nomen nudum* is unfounded. To our knowledge, Martens (1899) never introduced this taxon name, which therefore became a *nomen nudum* introduced by Counts (1991) himself.

oblonga Clessin, 1879

Corbicula oblonga Clessin, 1879: 267–268, pl. 36, fig. 18.

Type Locality: "Wahrscheinlich Australien"; Australia (?).

Type Material: Lectotype, **here designated**, ZMB 170.064a (pair of valves ex coll. Paetel; "*Corbicula oblonga* * Cless. 2 á 80 Orig Expl Cless 46.18!"; Fig. 2O), paralectotype ZMB 170.064b.

Comments: Clessin described this taxon on basis of the two shells from Paetel's collection – one of them is designated herein as the lectotype. The locality data has to be considered with caution because Paetel did usually not accurately file the information on the provenance of his material. So there is no indication if Australia should be considered as the origin of this material. Counts (1991: 27) refers to the misspelled name *C. oblongata* and states that the taxon should be considered to be a *nomen nudum*. This statement is misleading in respect to "*C. oblonga*", which is valid and available from a nomenclatural point of view, but it does hold true for the emended name introduced by Counts (1991) himself!

olivacea Krauss, 1848

Cyrena africana var. olivacea Krauss, 1848: 8–9.

Type Locality: "In flumine Gauritz, provincia Zwellendam (Krauss); in flumine Lepenula (Wahlberg); in parte superiore fluminis Nili (Parreyss)"; South Africa: Swellendam, Gauritz River; probably South Africa: Limpopo River; probably Sudan: Nile, upstream.

Type Material: Syntype ZMB 4.850 (two disarticulated valves; Gauritzfluss, leg. Krauss; Fig. 2P); syntype ZMB 28.486 (two disarticulated valves; Gauritzfluss, leg. Krauss); 2 syntypes ZMB 170.340 (two articulated valves and a single valve; Gauritzfluss, "*Cyrena gauritziana*"); syntypes SMF (not seen; partly referred to as types of *C. africana* by Counts (1991) and Daget (1998). Potential type material is also MCZ 154.102 (not seen), referred to as type of *C. africana* by Counts (1991) and Daget (1998). Note remarks under the latter species.

Comments: Krauss (1848) stated that he found not a single morphological characteristic to reliably distinguish between specimens collected by himself in the Gauritz River, specimens collected by Wahlberg in the Lepenula River, Inner Africa, that were obtained by Krauss from the museum in Stockholm, and the type specimens of *C. radiata* Philippi, 1846, as well as *C. pusilla* Philippi, 1846, both from the White Nile. Krauss (1848) stated that for the specimens collected by himself in the Gauritz River, he employed the manuscript name *C. gauritziana*. However, since this name was not published, it is not made available under the stipulations of the ICZN.

Krauss (1848) found only subtle differences in the shell size of all aforementioned specimens but recognized two different colour morphs: while the specimens from the Gauritz River were greenish, as were the specimens of *C. radiata*, those from the Lepenula River were either greenish as the former or yellowish-whitish as *C. pusilla* from the White Nile. Faced with the morphological similarity on one hand and considerable geographic distances between the different localities on the other hand, Krauss (1848) suggested that *C. pusilla*, *C. radiata*, the specimens collected by Krauss himself and those collected by Wahlberg altogether represented two varieties of the same species. Krauss (1848) employed the variety name "*olivacea*" for all greenish specimens and "*albida*" for all whitish specimens even though for both varieties already published names have been available, that is, *C. radiata* and *C. pusilla,* respectively, which were listed by Krauss (1848) in the synonymy of the new varieties. As a consequence, both variety names introduced by Krauss (1848) represent unnecessary replacement names for already existing names and therefore are permanently invalid. The name "*Corbicula africana* var. *olivacea* is an objective synonym of *C. radiata* and the name "*Corbicula africana* var. *albida*" is a synonym of *C. pusilla*. The specimen figured by Krauss (1848, pl. 1, fig. 8) is the only specimen to which the name *C. africana* explicitly was attributed by Krauss (1848) in the text reference to the illustration. Therefore, all specimens collected in the Gauritz River by Krauss, except for the one shell depicted in pl. 1, fig. 8, represent syntypes of one of the two varieties.

Because it is considered very difficult, if not impossible, to correctly identify the figured specimen among the series of specimens collected at this locality, even more so because further yet unidentified specimens may exist in other museum collections, we suggest that all former references to type material of *C. africana* in literature are in need of critical revision and instead likely refer to type material of one of the two varieties.

Counts (1991: 7) mentions paratype material of "*C. africana*" to be housed with the SMF and the MCZ. In contrast, Daget (1998: 168) considered the type specimen in the SMF as holotype with a paratype in the MCZ (154.182); note that the correct inventory number of this lot is MCZ 154.102, according to Adam J. Baldinger (pers. comm.). In addition, this lot contains two different labels with contradictory information: One label states "Gauritz River, Swellen dam, Cape Province", while the other label reads "Cape of Good Hope, coll. Krauss" The second label seems to be the original one, suggesting that the material associated with it most likely has no type status.

We were not able to compare the types reported in literature, that is, by Counts (1991) and Daget (1998) with the illustration of Krauss, but are sure that the specimens housed with the ZMB do not perfectly match this figure. For the time being we consider it very likely that also the specimens in the SMF represents syntype material only of one of the two varieties, and not the holotype of *C. africana*.

Daget (1998: 168) mentions further types of "var. *olivacea*" in the SMF, but this material could not be traced (Ronald Janssen, pers. comm.).

oxiana Martens, 1876

Cyrena (Corbicula) fluminalis var. oxiana Martens, 1876: 337.

Corbicula oxiana – Martens, 1882: 48, pl. 4, fig. 15.

Type Locality: “Chiwa” More precise data was given by Martens (1882): “Turkestan …ehemaligen Chanate Chiwa … im ausgetrockneten Bett eines Arms des Amu-Darja zwischen Scheich-Dsheili und Kalendar-chana”; i.e. Uzbekistan: dry river bed of a tributary of the Amu-Darja in the area of Khiva (41°22’N, 60°21’E) between Sheikh-Dsheili and Kalendar-chana. This location is near Urgench; the correct spelling of the local names remains unclear.

Type Material: Lectotype, **here designated**, ZMB 27.360b (pair of valves; “*Cyrena fluminalis* var. *oxiana* Martens *, ~~Chiwa~~ Amu-Darja”, “*Cyrena fluminalis* Mll. var. *oxiana* Mts., ausgetrocknetes Bett eines Arms des Amu Darja zwischen Scheich Dscheil und Kalendar chana”; Fig. 3F); paralectotype ZMB 27.360a (left valve); paralectotypes ZMB 170.034 (pair of valves and right valve; “flum var Oxiana Scheich Dscheil”).

Comments: The specimen figured in Martens (1882) is herein designated as the lectotype. Counts (1991: 18) mentions an incorrect inventory number (“27.361”) of the type material referring to the “holotype”. However, the description of Martens (1876) is not explicitly based on only a single specimen. The type series was separated, because one of the shells was temporarily placed in the exhibition of the ZMB. When in 1970 it was returned to the Malacological collection, it has not been re-lumped with the other type specimens but remained as a separate lot (ZMB 27.360b).

The locality “Chiwa” [= Khiva or Chiva] given by Martens (1876) refers to a larger area, while the information given subsequently by Martens (1882) seems to refer to the exact locality, which is situated in the wider area of Khiva City. The label data has subsequently been corrected into “Amu-Darja”, which refers to the largest river in this area and fits to the specification given by Martens (1882).

Some Russian authors (e.g., Izzatullaev 1980) regarded *C. oxiana* to be synonym of *C. purpurea* Prime, 1864; however, the figure given by the latter author for *C. purpurea* shows a shell rather different from the *oxiana* type. Kinzelbach (1992), while not mentioning *C. oxiana,* noted that *C. fluminalis* lives in the area of the Aral basin, where the type locality is (Fig. 1, loc. 7). Korniushin (2004) argued that *C. purpurea* should be synonymized with *C. fluminalis*.

possoensis P. Sarasin & F. Sarasin, 1898

Corbicula possoensis P. Sarasin & F. Sarasin, 1898: 92–93, pl. 11, figs. 161–162.

Type Locality: “Posso-See”; Indonesia, Sulawesi: Lake Poso (1°47’54"S, 120°35’56"E).

Type Material: Syntype ZMB 50.798 (pair of valves; “*Corbicula possoënsis* SS, S Celebes, Posso See, Sarasin’s S.G.”; Fig. 3G).

Comments: The type material has been obtained by the museum from the Sarasin cousins. Further types are deposited with the museum in Basel (MHNB 42A), according to Counts (1991: 30).

producta Martens, 1905

Corbicula producta Martens, 1905: 66, pl. 2, fig. 8.

Type Locality: “Keumgang bei Kongju, Provinz Chhungchhöngdo, Korea”. South Korea: Prov. Ch’ungch’ong-namdo, Keum River near Kongju (36°28’N, 127°06’E).

Type Material: Lectotype, **here designated**, ZMB 55.625a (pair of valves; “*Corbicula producta* Marts.* Keumgang”; Fig. 3E); paralectotypes ZMB 55.625b (three single valves); paralectotypes ZMB 38.431 (2 pair of valves, 3 right valves; “*Corbicula* no. 3, 11/8 Keumgang, 10 Li SO of Kongju, Gottsche”).

Comments: The specimen ZMB 55.625 represents the shell figured in the original description and is herein designated as the lectotype. It was erroneously referred to as “holotype” by Counts (1991: 30). However, Martens did not explicitly state that his description is based on this specimen only. In the ZMB collection catalogue under the entry “ZMB 38.431” is noted “5 *Corbicula* Keumgang”, referring to five complete specimens that constitute the type lot. Of these specimens, three right valves (ZMB 38.431) perfectly fit the left valves of ZMB 55.625. We assume, therefore, that the five specimens of lot ZMB 38.431 were separated subsequently and placed into two different boxes, one of them ZMB 55.625.

pullata Philippi, 1850

Cyrena pullata Philippi, 1850: 110.

Type Locality: "Sumatra, Danau Luar".

Type Material: Lectotype, **here designated**, ZMB 170.457a (pair of valves; "Sumatra, Danau Luar, leg. Koch, ex coll. Dunker"; Fig. 4A); paralectotype ZMB 170.457b (pair of valves).

Comments. The material originates from the collection of Wilhelm Dunker (1809–1885). Dunker obtained this material from R. A. Philippi (1808–1904), as can be judged from the label data; see also comments of Martens (1897: 117–118).

We are not able to trace a locality with that (or a similar) name in Sumatra. Danau Luar is located in central west Borneo (0°56'N, 112°14'E). Therefore, it remains to be clarified whether the types indeed originate from Sumatra.

regia Clessin, 1879

Corbicula regia Clessin, 1879: 267, pl. 43, fig. 5.

Type Locality: "Wahrscheinlich Indien"; likely India; in error for Penang, Malaysia (see Comments below).

Type Material: Lectotype, **here designated**, ZMB 170.404a (pair of valves ex coll. Paetel; "*Corbicula regia* Bens Penang 30", added in Martens' handwriting "Cless. 43.5!"; Fig. 2Q); paralectotype ZMB 170.404b (right valve).

Comments: The name was attributed to Benson by Clessin (1879: "*Corb. regia* Bens. in Coll. Paetel"), but Benson did not describe this taxon. The provenance of the material is not clear. What is clear, on the other hand, is that it originates from the Paetel collection along with the manuscript name of Benson. Because Clessin (1879) is the first author who published a description for this taxon, he is also the author of the name *C. regia*. Prashad (1928) states that *C. regia* is not an Indian species but that the series from which most likely also Paetel's material originates came from Penang (Malaysia). This fits to the data on the original label of Paetel. Consequently, the statement "likely India" given by Clessin (1878) should be considered erroneous.

sandai Reinhardt, 1878

Corbicula sandai Reinhardt, 1878: 187–189, pl. 5, fig. 2.

Type Locality: "Kioto, Japan (von Herrn Rein)"; Japan: Kyoto (35°00'N, 135°45'E).

Type Material: Syntypes ZMB 29.273 (3 pairs of valves; "*Corbicula sandai*, Kioto, Japan, [leg.] Rein"; Fig. 4B).

Comments: This species was described for a large series of specimens collected by Johannes Justus Rein (1835–1918) during his travels in Japan in 1873–1875. Subsequently, parts of this series were apparently distributed to various collections. Consequently, additonal type material may be deposited in other museums. Since the description of Reinhardt (1878) is not explicitly based on a single specimen, we disagree with the report of a holotype to be held by the SMF (Counts 1991: 32). Instead we consider the material in the SMF as representing syntype material (SMF 6007-8; not seen (Counts, 1991; Morton, 1986).

Several reproductive biological features (e.g., monoflagellate spermatozoa, oviparity) distinguish this taxon from most other known Southeast Asian *Corbicula* species, for example, from Japan, Korea, Thailand and Indonesia, as well as from Australia; see, for example, literature in Glaubrecht et al. (2006). Based on mitochondrial sequence data (COI), *C. sandai* represents a distinct clade, endemic to Lake Biwa in Japan (Siripattrawan et al., 2000) which, however, shows affinities to those taxa endemic on the island of Sulawesi (Glaubrecht et al., 2003; Rintelen & Glaubrecht, 2006; Glaubrecht et al., 2006).

sayana Prime, 1864

Corbicula sayana Prime, 1864: 71–72, fig. 19.

Type Locality: "Insulae Philippinenses. Collect Wheatley and Prime"; Philippines.

Type Material: Syntypes ZMB 32.199 (3 pairs of valves ex coll. Gundlach; "*Corbicula sayana* Prime, Philippinen, Prime"; Fig. 4C).

Comments: According to Johnson (1959: 471) and Counts (1991: 32), the holotype is deposited in the MCZ (MCZ 135631). However, Prime did not explicitly designate a holotype, and his description apparently is based on more than a single specimen. The mentioning of a holotype by Johnson (1959) and Counts (1991) probably reflects that only a single specimen was available to them. For that reason, we consider also the specimen of the MCZ to be a syntype.

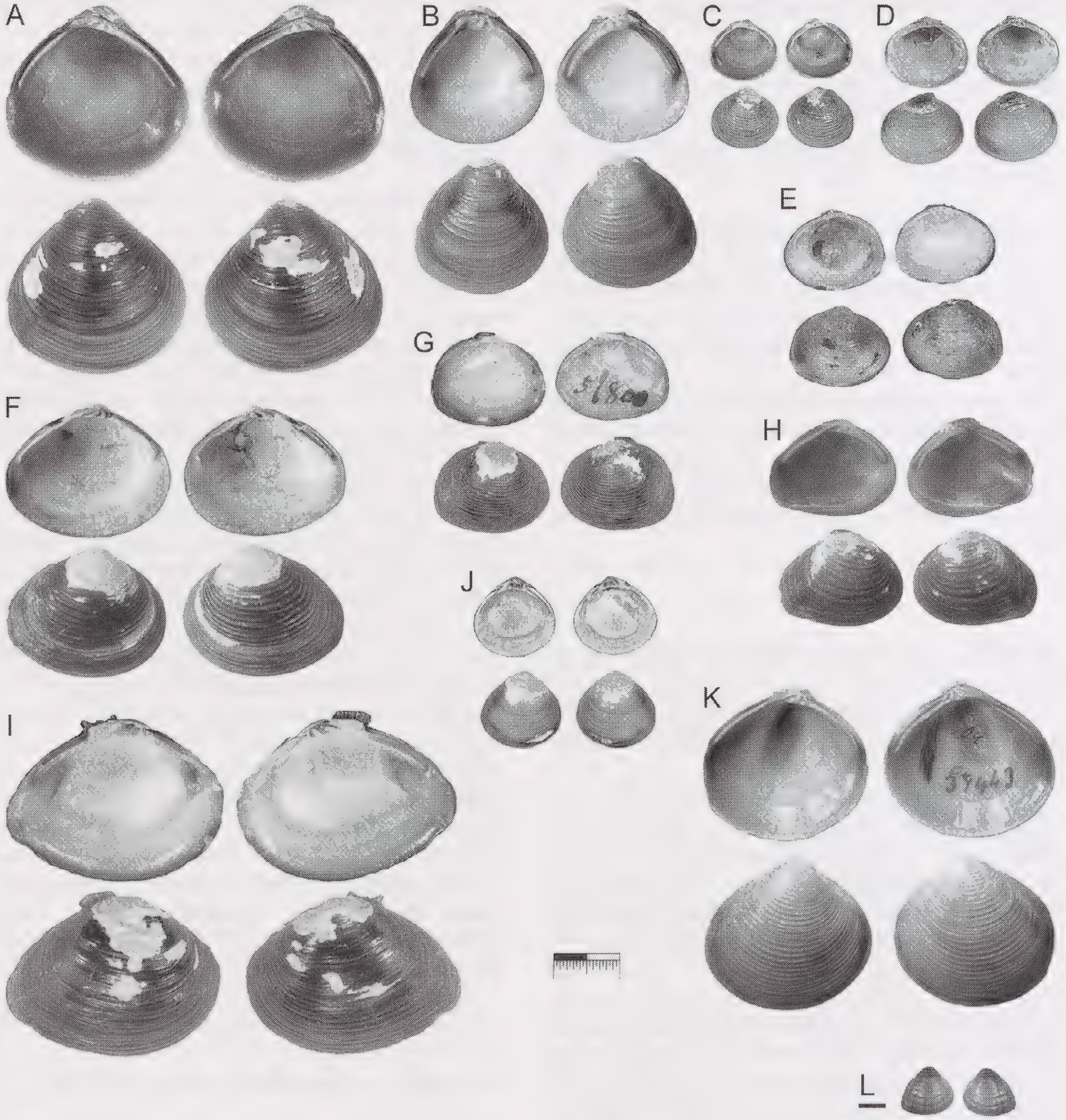


FIG. 4. Type Material of corbiculids from ZMB collection (natural size), continued. A: Lectotype of *Cyrena pullata* Philippi, 1850 (ZMB 170.457a; Indonesia, Sumatra: Lake Luar); B: Syntype of *Corbicula sandai* Reinhardt, 1878 (ZMB 29.273; Japan: Kyoto); C: Syntype of *Corbicula sayana* Prime (ZMB 32.199; Philippines); D: Paralectotype of *Corbicula straminea* Reinhardt, 1877 (ZMB 28.124; Japan, Lake); E: Topotypical specimens (potential syntypes; see text) of *Corbicula sikorae* Ancey, 1890 (ZMB 45.446; Madagascar: close to Antananarivo, at an elevation of 700 m); F: Lectotype of *Corbicula subplanata* Martens, 1897 (ZMB 103.017a; Indonesia, Sulawesi: Minralang); G: Lectotype of *Corbicula tobae* Martens, 1900 (ZMB 51.800a; Indonesia, Sumatra, Lake Toba); H: Lectotype of *Cyrena* (*Corbicula*) *transversa* Martens, 1877 (ZMB 26.394a; Japan: Yokohama); I: Syntype of *Corbicula trapezoidea* Martens, 1897 (ZMB 170.006; Indonesia, Sumatra: Lake Diatas); J: Lectotype of *Corbicula tsadiana* Martens, 1903 (ZMB 53.631; Chad: Lake Chad, S shore); K: Holotype of *Corbicula zelebori* Jickeli, 1874 (ZMB 37.443; Egypt: near Suez, in freshwater canal). Scale bar = 10 mm. L: Syntype of *Corbicula cochinensis* Preston, 1916 (ZMB 64.717; India: Kerala, Kochi backwater near Ernakulam). Figure 4 x enlarged. Scale bar = 1 mm.

Prashad (1930) considered *C. sayana* to be a synonym of *C. manilensis*, which he regarded as "allied to *C. fluminea*", which is widespread in SE Asia; see also Morton (1977).

sikorae Ancey, 1890

Corbicula sikorae Ancey, 1890: 345.

Type Locality: "Fleuve Mangoro, dans l'intérieur de Madagascar, de Tananarive á la côte orientale, á une altitude de 700 métres au-dessus du niveau de la mer (Sikora)"; Madagascar: close to Antananarivo (18°53'S, 47°30'E), at an elevation of 700 m.

Type Material: Topotypic material (ZMB 45.446; 2 adult specimens; "2 Schalen von *Corbicula sikorae* Anc. n. sp., Madagaskar Sikora r."; Fig. 4E).

Comments: This ZMB material, collected at the type locality, was catalogued in the ZMB in 1892 and was obtained from the Austrian collector Franz Sikora (?–1902), who was based in Réunion but collected also for seven years for various European museums collections on Madagascar. Therefore, it is concluded that the present specimens represent topotypical material from Sikora's original series. However, it is not sure whether they were also available to Ancey (1890), who described the species. Only then they would truly represent syntypes. However, it could well be that the original series has been split before Ancey has received material from Sikora, and then curator in the ZMB, Eduard von Martens (1832–1904), only later, when cataloguing the lot, noted (see the original label) that Ancey has described this to represent a new species. Therefore, the status of the material in the ZMB is uncertain. Daget (1998) and Counts (1991) were not able to trace Ancey's type material of this taxon. Since the size parameters given in Ancey's original description (L: 11.5, H: 8.5, W: 5 mm) do not correspond with neither of the two ZMB specimens (Glaubrecht et al., 2006) and we have no further information as to any potential relationship between Ancey, Sikora and Martens, we refrain from assuming this material to actually represent types, but instead regard it to represent topotypical material, collected by Sikora together with Ancey's later type material. It should be noted, though, that in case Ancey's types are evidently lost, the ZMB material qualifies for the designation as neotypes.

Corbicula sikorae Ancey, 1890, is treated as junior synonym of *C. madagascariensis* Smith, 1882 in the most recent catalogue of African bivalves (Daget 1998). Conchological examination of the two specimens (ZMB 45.446) and their comparison with Ancey's (1890) and Smith's (1882) original descriptions, support the proposed synonymy. Clusters of young specimens at the inner side of the adult shells indicate that this is a brooding species with a potential euviviparous mode; see details in Glaubrecht et al. (2006).

straminea Reinhardt, 1877

Corbicula straminea Reinhardt, 1877: 70, pl. 5, fig. 1.

Type Locality: not given by Reinhardt (1877). Lake Hakone, Japan, designated as the type locality (see Counts 1991: 34); probably Hakone: Lake Ashi (Ashino-ko), Honshu, Kanagawa Pref. (35°24'N, 139°03'E).

Type Material: Lectotype, **here designated**, SMF 6006 ("Japan, Hakone-See, ex Dönitz); paralectotypes SMF 6004/2 ("Japan, Hakone-See, ex Dönitz"); paralectotypes SMF 6005/5 ("Saga, Insel Kiushiu, ex Dönitz"); paralectotypes ZMB 28.124 (4 pairs of valves; "*Corbicula straminea* Reinh. * Hakone See, Hilgendorf Dönitz").

Comments: Reinhardt (1877) gave a shell size of 13 x 10.5 mm, which fits quite well with the size of the SMF specimen (13.1 x 11.2 mm; R. Janssen, pers. comm.), which is here designated as the lectotype. In a later report, Reinhardt (1878: 186–187) published a description along with a figure that is based on additional material collected by Johannes Justus Rein. This material, however, was not available to Reinhardt when he described this taxon in 1877, and therefore is not qualified as types. In his description, Reinhardt (1877: 70) did not give the type locality. According to the label data Lake Hakone, Japan, was designated as type locality of *C. straminea*.

Note that Prashad (1924: 528) said he had examined the ZMB material in this case; in contrast to Pilsbry (1907), he considered this taxon to be distinct from *C. leana* Prime, 1864.

subplanata Martens, 1897

Corbicula subplanata Martens, 1897: 112, pl. 7, figs. 7–10.

Type Locality: "Celebes: Fluss Minralang bei Tempe"; Indonesia, Sulawesi: Walanae River near Tempe (4°11'S, 120°04'E).

Type Material: Lectotype, **here designated**, ZMB 103.017a (pair of valves; "*Corbicula subplanata* Marts.*, Minralang, Celebes, Weber"; Fig 4F); paralectotypes ZMB 103.017b (5 pairs of valves, 1 left valve; "*Corbicula subplanata* Marts.*, Minralang, Celebes, Weber").

Comments: The specimen ZMB 103.017a is marked with an asterisk at the inner side of the valves, which usually indicates that the specimen was selected for drawing. It represents the specimen figured in Martens (1897: pl. 7, figs. 9–10), as can be recognized by particular corrosion on the umbo; it also corresponds to the published measurements (L = 25 mm, H = 20 mm, W = 13 mm) and is, thus, here designated as the lectotype. Counts (1991: 34) was not able to trace type material.

Djajasasmita (1975, 1977) suggested synonymy of *C. celebensis* with *C. subplanata*. His proposal, followed here, is supported by recent molecular evidence (Rintelen & Glaubrecht, 2006; Glaubrecht et al., 2006) insofar as all riverine *Corbicula* on Sulawesi (to the exclusion of *C. linduensis*, i.e. those classified in the past as either *C. subplanata* or *C. celebensis* on conchological grounds only) form one closely allied genetic cluster.

tobae Martens, 1900

Corbicula tobae Martens, 1900: 17.

Type Locality: "See Toba bei Tongging"; Indonesia, Sumatra, Lake Toba (2°36'N, 98°56'E).

Type Material: Lectotype, **here designated**, ZMB 51.800a (pair of valves; "*Corbicula tobae* n. sp., N Sumatra, Toba See bei Tongking, G. Schneider"; Fig. 4G); paralectotypes ZMB 51.800b (two pairs of valves); paralectotypes ZMB 51.801 (2 left, 2right valves (two specimens?); "*Corbicula tobae* Martens, Toba See, N Sumatra").

Comments: The largest shell of the lot is herein designated as the lectotype. Counts (1991: 36) was not able to trace types, but mentioned topotypic material in the MHNB (MNHB 46A).

transversa Martens, 1877

Cyrena (*Corbicula*) *transversa* Martens, 1877: 120.

Type Locality: "Yokohama, Japan" (35°26'N, 139°39'E).

Type Material: Lectotype, **here designated**, ZMB 26.394a (pair of valves; "*transversa* Marts* = *ovata* Prime Risch", "*Corbicula* ~~*ovata*~~ *transversa* Mart *, SB. nat Fr. 1877. = *ovalis* Prime Rurh Jahrl 1878 5 5, Yokohama v. Martens", "*C. ovalis* Prime (*C. transversa* v Mart) Yokohama v. Martens", "*Corbicula dönitziana* Clessin monogr p 197 39 Fig 4 = var... *transversa* 38, 13"; Fig. 4H); paralectotype ZMB 26.394b (5 pairs of valves); paralectotypes SMF 6019 (not seen; fide Counts 1991: 37).

Comments: The largest shell of the lot is herein designated as the lectotype. Counts (1991: 37) referred to the lot SMF 6019 as paratype material. However, in his description, Martens (1877) did not explicitly mention a holotype; thus, the types in the SMF cannot be considered as representing paratypes. In the collection of the ZMB there are additional lots of *Corbicula* from Yokohama that were also collected by Martens. Their labels show that apparently Martens had trouble in satisfactorily distinguishing the different taxa, as is evident from such redundant naming as, for example, "*C. transversa*, *cuneata*, *ovalis*" for lot ZMB 170.414, "*C. martensii* Cless., *fuscata* var. *atrata* Reinh." (ZMB 17.456), and "*C. biformis* Reinh., *japonica* Prime" (ZMB 26.550).

Prashad (1924) stated that *C. transversa* is a distinct species, albeit allied to *C. leana*. It should be noted that colour and sculpture resembles that of *C. japonica*, a widespread brackish-water *Corbicula* species. In addition, the type locality Yokohama is a major port, rendering it possible that the *C. transversa* specimens originated from a brackish-water population of *C. japonica*.

trapezoidea Martens, 1897

Corbicula trapezoidea Martens, 1897: 115–116, pl. 7, figs. 14–19.

Type Locality: "Sumatra: im See Danau di atas"; Indonesia, Sumatra: Lake Diatas (01°05'S, 100°45'E).

Type Material: Syntypes ZMB 170.006 (4 articulated pairs of valves, 1 left valve; "*Corb.*

trapezoidea, Danau di atas"; Fig 4I); syntypes ZMB 170.007 (23 articulated pairs of valves, 1 left valve, 1 right valve; "*Corbicula trapezoidea*, Danau di atas, Sumatra"); syntype ZMB 170.462 (juvenile specimen; three labels read "*trapezoidea* juv", "VII fig 19" and "See Manindjau").

Comments: The lot ZMB 170.462 contains a juvenile specimen; the labels indicate that this specimen is depicted in pl. 7, fig. 19 (Martens, 1897). Consequently, this specimen is regarded as a syntype, although if the third label is correctly placed here it apparently does not originate from the type locality, as given by Martens (1897).

tsadiana Martens, 1903

Corbicula tsadiana Martens, 1903a: 9–10.

Type Locality: "Südufer des Tschad-Sees"; Chad: Lake Chad, S shore (12°47'N, 14°33'E).

Type Material: Lectotype, **here designated**, ZMB 53.631 (pair of valves; "*Corbicula tsadiana* Marts*, Tsad-see Südufer, Glauning S"; Fig. 4J); paralectotype ZMB 55.359 (7 single valves; "*Corbicula tsadiana* Marts. Südufer des Tsadsees Glauning", not in Martens' handwriting.

Comments: The largest specimen is designated as the lectotype. Counts (1991: 37) referred to the specimen ZMB 53.361 as holotype. However, the original description is not explicitly based on a single specimen.

Daget (1998) listed *C. tsadiana* as synonym of *C. consobrina*, whereas Mandahl-Barth (1988) treated it as a local race of *C. fluminalis*, stating that "it is connected with that form through gradual transitions, [while] more normal, *consobrina*-like forms are also found in the lake [Lake Chad]".

zelebori Jickeli, 1874

Corbicula zelebori Jickeli, 1874: 290, pl. 9.

Type Locality: "im Süsswasser-Kanal bei Suez"; Egypt: near Suez (29°57'N, 32°34'E), in freshwater canal.

Type Material: Holotype ZMB 37.443 (pair of valves; "*Corb. Zelebori* Jick., Suez Süsswasser canal, Jickeli"; Fig. 4K).

Comments: Jickeli (1874) explicitly based his description on a single specimen, which is consequently the holotype. The name was misspelled "*zelibori*" by Counts (1991: 39).

Both Daget (1998) and Mandahl-Barth (1988) treated *C. zelebori* as synonym of *C. consobrina*.

Batissa Gray, 1853

angulata Reinhardt, 1886

Batissa angulata Reinhardt, 1886: 62–63.

Type Locality: "Neu-Guinea, Angriffshafen"; Papua New Guinea: northern coast near Vanimo (2°40'S, 141°18'E).

Type Material: Lectotype, **here designated**, ZMB 38.312a (pair of valves; "*Batissa angulata* Reinh. SB nat. Fr. 1886 p. 62, Angriffshafen, Neu Guinea, Finsch"; Fig. 5A); paralectotypes ZMB 38.312b (3 pairs of valves).

Comments: The largest specimen is herein designated as the lectotype. Reinhardt (1886: 63) gave shell parameters (length = 110, height = 93, width = 57 mm), which do not corresponds to the lectotype.

celebensis Martens, 1897

Batissa violacea var. *celebensis* Martens, 1897: 104–105, pl. 5, fig. 3.

Type Locality: "Celebes: Tjenrana. Fluss bei Pampanua im Gebiet von Boni.... Fluss bei Maros (unweit Makassar)"; Indonesia: Sulawesi, restricted here to Tjenrana, river near Pampanua in the vicinity of Boni (4°30'S, 120°31'E). Locality of paralectotypes: River near Maros.

Type Material: Lectotype, **here designated**, ZMB 111.986 (pair of valves; "*Batissa violoacea* Brug. Pampanua Fluss Tjinrana. Celebes. M. Weber"; Fig. 5B); paralectotypes ZMB 111.985 (3 pairs of valves, single valve; "*Batissa violacea* var. *celebensis*, Fluss bei Maros"); paralectotype ZMB 54346 ("Sulawesi, leg. Weber"; soft body in ethanol, marked as type).

Comments: The specimen depicted in the original description (pl. 5, fig. 3) is herein designated as the lectotype.

discoidea Martens, 1897

Batissa violacea var. *discoidea* Martens, 1897: 106, pl. 5, fig. 9.

Type Locality: "Celebes. Fluss bei Maros": Indonesia: Sulawesi, river near Maros (5°00'S, 119°36'E).

Type Material: Holotype ZMB 111.987 (pair of valves; "*Batissa violacea* var. *discoidea*, Fluss bei Maros, Celebes, M. Weber"; Fig. 5C).

Comments: In his description Martens (1897: 107) explicitly states "Ein Exemplar" [one specimen], which renders the specimen kept in the ZMB the holotype. It represents the specimen figured in pl. 5, fig. 9, as can be judged from comparison.

extensa Martens, 1897

Batissa violacea var. *extensa* Martens, 1897: 106–108, pl. 5, fig. 7.

Type Locality: "Celebes: Fluss bei Maros": Indonesia: Sulawesi, river near Maros (5°00'S, 119°36'E).

Type Material: Holotype ZMB 109.026 (pair of valves; "*Batissa violacea* var. *extensa* Marts. Maros Celebes"; Fig. 5D).

Comments: In his description, Martens (1897: 107) explicitly states "Ein Exemplar" [one specimen], which renders the specimen kept in the ZMB the holotype. It represents the specimen figured in pl. 5, fig. 7, as can be judged from comparison.

finschi Reinhardt, 1886

Batissa finschi Reinhardt, 1886: 61.

Type Locality: "Nordküste von Neu-Guinea, Venus Point, etwa gegenüber der Vulcan-Insel"; Papua New Guinea: Northern coast, Venus Point, opposite of volcanic island (4°02'S, 144°41'E).

Type Material: Lectotype, **here designated**, ZMB 38.314a (pair of valves; "*Batissa finschi* Reinh. SB nat. Fr. 1886 p. 61*, Venus Hoek, Neu Guinea, Finsch"; Fig. 5E); paralectotypes ZMB 38.314b (2 pairs of valves).

Comments: The largest specimen of the lot is herein designated as the lectotype.

macassarica Martens, 1897

Batissa violacea var. *macassarica* Martens, 1897: 105–106, pl. 5, fig. 8.

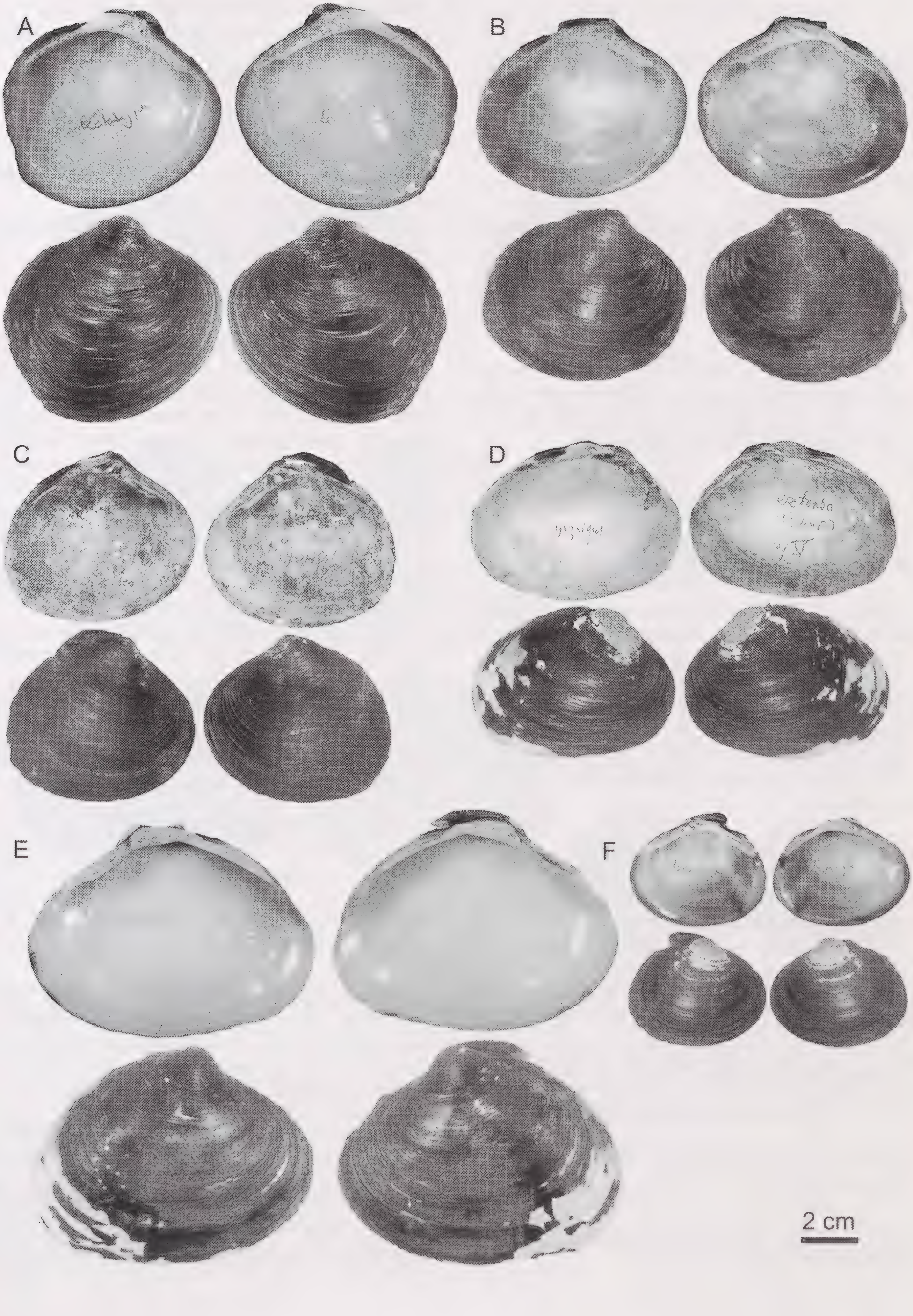
Type Locality: "Celebes: Makassar, v. Martens, 1862": Indonesia: Sulawesi, Macassar (5°09'S, 119°26'E) (leg. Martens, 1862)].

Type Material: Lectotype, **here designated**, ZMB 108.824a (pair of valves; "*Batissa macassarica* Marts. * Makassar Martens"; Fig. 5F); paralectotypes ZMB 108.824b (2 pairs of valves).

Comments: The type series consists of three specimens. The largest shell, which was depicted in the original description (Martens, 1897: pl. 5, fig. 8), is herein designated as the lectotype.

→

FIG. 5. Type material of corbiculids from ZMB collection (half of natural size), continued. A: Lectotype of *Batissa angulata* Reinhardt, 1886 (ZMB 38.312a; Papua New Guinea: Northern coast near Vanimo); B: Lectotype of *Batissa violacea* var. *celebensis* Martens, 1897 (ZMB 111.986; Indonesia: Sulawesi, Tjenrana, river near Pampanua in the vicinity of Boni); C: Holotype of *Batissa violacea* var. *discoidea* Martens, 1897 (ZMB 111.987; Indonesia: Sulawesi, river near Maros); D: Holotype of *Batissa violacea* var. *extensa* Martens, 1897 (ZMB 109.026; Indonesia: Sulawesi, river near Maros); E: Lectotype of *Batissa finschi* Reinhardt, 1886 (ZMB 38.314a; Papua New Guinea: northern coast, Venus Point, opposite of volcanic island); F: Lectotype of *Batissa violacea* var. *macassarica* Martens, 1897 (ZMB 108.824; Indonesia: Sulawesi, Macassar). Scale bar = 20 mm.


A
B
C
D
E
F
2 cm

schmidti Martens, 1903

Batissa schmidti Martens, 1903b: 426–427, text-fig.

Type Locality: “Kari-Orang, Ost-Borneo”; Indonesia: Borneo, Kalimantan Timur, Kariorang (0°50’S, 117°55’E).

Type Material: Lectotype, **here designated**, ZMB 108.825a (pair of valves; “*Batissa*, Kari Prang, SO Borneo, [leg.] M. Schmidt”; Fig. 6A); paralectotypes ZMB 108.825b (5 pairs of valves).

Comments: The largest specimen is herein designated as the lectotype, it represents the specimen depicted in the (enlarged) original figure (Martens, 1903b: 427).

subtrigona Thiele, 1928

Batissa subtrigona Thiele: 1928: 144–145, text-fig.

Type Locality: “Fluss Mamberamo, West Neu-Guinea”; Indonesia: Irian Jaya: Mamberamo River (2°06’S, 137°47’E).

Type Material: Lectotype, **here designated**, ZMB 111665a (pair of valves; “West New Guinea: Mamberamo River, leg. Mozkowski”; Fig. 6B); paralectotype ZMB 111665b (pair of valves).

Comments: The description was explicitly based on two specimens (Thiele, 1928: 144). The schematic text figure gives only the outline of the shell, but it is not to scale. Since both specimens are very similar to each other in size and shape, we cannot identify the specimen on which the figure is based. The specimen figured herein is designated as the lectotype.

Polymesoda Rafinesque, 1820

cordata Martens, 1865

Cyrena cordata Martens, 1865: 65–66, ex Wiegmann MS.

Type Locality: “Mexiko”; Mexico: more precise location unknown.

Type Material: Holotype ZMB 17.450 (pair of valves; “*Cyrena cordata* Wiegm.* Mexiko, [leg.] Deppe”; Fig. 6D).

Comments: When describing the new taxon, Martens was dealing with a single specimen, as can be concluded from the measurements he gave referring to one specimen. The taxon is not listed in Kabat & Boss (1997).

The current systematic placement under *Polymesoda* remains to be verified.

Incertae Sedis

The following corbiculid taxa are listed with their original generic assignment, because their actual phylogenetic relationships remain to be verified. The Indonesian “*Cyrena*” *prona* (Fig. 6E) and “*Cyrena*” *moluccensis* (Fig. 6C) differ in conchological characters from typical *Corbicula*, but resemble taxa assigned to the Asian *Geloina* Gray, 1840, the type species of which is *Geloina coaxans* (Gmelin, 1791). However, the status of *Geloina*, treated at times as a subgenus of the New World *Polymesoda*, is in need of clarification.

It should be noted again that the name *Cyrena* Lamarck, 1818, as applied by earlier authors particularly for African taxa (e.g., Philippi 1846–1850; Krauss, 1848; Martens 1897), is considered to be a junior synonym of *Corbicula*; see, for example, Pilsbry & Bequaert (1927).

Cyrena Lamarck, 1818

prona Martens, 1908

Cyrena prona Martens, in Martens & Thiele, 1908: 289–290, pl. 6, fig. 24.

Type Locality: “Kalkbach bei Tandjong batu pondong am Gunung Sekerat”; Indonesia: Kalimantan Timur (= Eastern Borneo), creek near Tandjung Batu Pondong, Gunung Sekerat (0°52'S, 117°48'E).

Type Material: Lectotype, **here designated**, ZMB 108.823a (pair of valves; “*Cyrena prona* n., Ost Borneo Mart. Schmidt [handwriting of Martens”, “*Cyrena prona* Marts. * Ost Borneo, M. Schmidt, 19.2.1902”; Fig. 6E); paralectotypes ZMB 108.823b (2 pairs of valves).

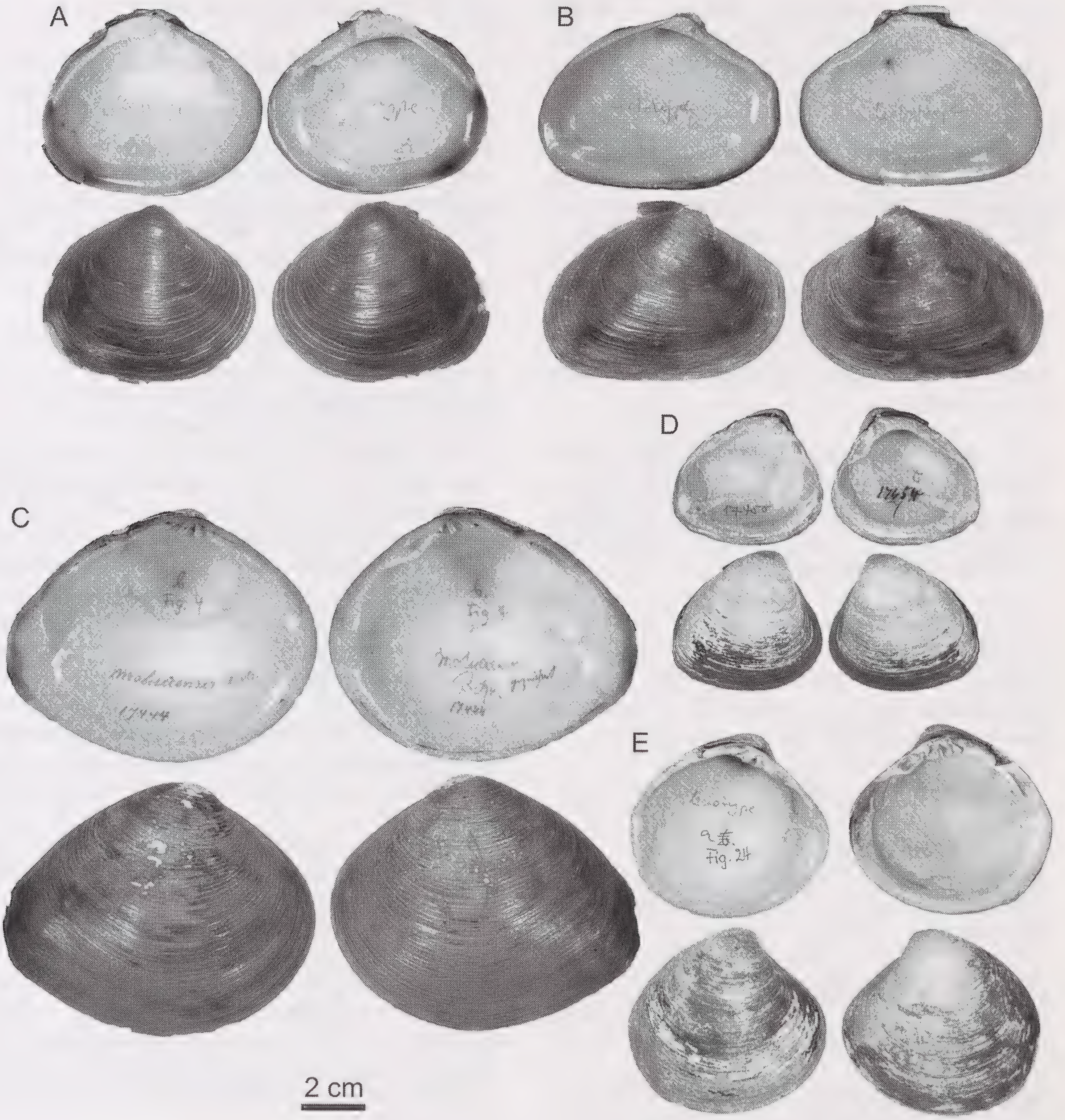


FIG. 6. Type material of corbiculids from ZMB collection (half of natural size), continued. A: Lectotype of *Batissa schmidti* Martens, 1903 (ZMB 108.825a; Indonesia: Kalimantan Timur, Kari Orang); B: Lectotype of *Batissa subtrigona* Thiele, 1928 (ZMB 111665a; Papua New Guinea: Mamberamo River); C: Lectotype of *Cyrena moluccensis* Martens, 1897 (ZMB 17.444a; Indonesia: Moluccas: Batjan); D: Holotype of *Cyrena cordata* Martens, 1865 (ZMB 17.450; Mexico); E: Lectotype of *Cyrena prona* Martens in Martens & Thiele, 1908 (ZMB 108.823; Indonesia: Kalimantan Timur, creek near Tandjung Batu Pondong, Gunung Sekerat). Scale bar = 20 mm.

Comments: The specimen figured in the original description, as can be judged from the comparison of the illustration, is designated herein as the lectotype for the stabilization of the name.

moluccensis Martens, 1897

Cyrena moluccensis Martens, 1897: 96–97, pl. 6, fig. 4.

Type Locality: “Molukken: Batjan, in einem Morast, der nur durch einen schmalen Landstreifen vom Meer getrennt, in Gesellschaft mit *C. oaxans*, v. Martens, 1862; Ternate, eine jüngere Schale, v. Martens; Ceram, bei Pasahari, Martin, 1892; Molukken, ohne nähern Fundort, Semper bei Clessin, möglicher Weise von mir stammend”. Indonesia: Moluccas: herein restricted to Batjan (8°33’S, 116°22’E), peat near the coast, separated from the open sea only by a narrow stretch of land, together with *C. oaxans* (leg. Martens, 1862). Localities of paralectotypes: Ternate (leg. Martens); Seram, near Pasahari (leg. Martin).

Type Material: Lectotype, **here designated**, ZMB 17.444a (pair of valves; “*Cyrena* ~~*expansa*~~ ~~Mss~~. *moluccensis* Martens * Batjan, Molukken, v. Martens”; Fig. 6C); paralectotypes ZMB 17.444b (6 pairs of valves).

Comments: In his description, Martens (1897) referred to a number of differently sized and shaped shells (termed “a–g”). The specimen depicted in Martens (1897: pl. 6, fig. 4) is herein designated as the lectotype.

Villorita Gray, in Griffith and Pidgeon, 1834

cochinensis Preston, 1916

Corbicula cochinensis Preston, 1916: 36, fig. 12, 12a, b.

Type Locality: “Cochin backwater, near Ernakulam”; India: Kerala, Kochi backwater near Ernakulam (10°00’N, 76°13’E).

Type Material: Syntypes ZMB 64.717 (2 pairs of valves; “*Corbicula cochinensis* Preston, Cochin W. India, ded. Preston”; Fig. 4L).

Comments: The material in the ZMB has been obtained from Fulton as “cotype”. Counts (1991: 12) did not mention type material.

Prashad (1928: 17) stated that Preston’s description of *C. cochinensis* was presumably based on young shells of *Villorita cyprinoides* Gray, 1834. The assignment to *Corbicula* or alternatively to *Villorita* remains to be verified; the status of the latter as a genus distinct from the former is unresolved.

CONCLUSIONS

A modern and comprehensive classification based on molecular and/or morphological cladistic analyses is still lacking for Corbiculidae. We anticipate that the present catalogue, providing baseline nomenclatural and taxonomic data from one of the major collections of this limnic bivalve group, will eventually facilitate the systematic revision of these ecologically important and evolutionarily fascinating molluscs.

Most of the taxa documented here are still poorly known and do not belong to the few invasive lineages that have attracted so much attention in recent years. Recent studies, however, showed that at least the invaders display very complex, not yet fully understood genetic structures, which renders taxonomic decisions and phylogenetic examinations extremely difficult. For instance, especially invaders of the New World and Western Europe, display a wide range of distinct morphotypes (now found on multiple continents) that share identical mt haplotypes (Pfenninger et al., 2002; Lee et al., 2005). Similarly, it has been shown for “indigenous” Asian Corbiculidae that lineages characterized by distinct nuclear genomes share identical mt haplotypes (Park et al., 2002).

Traditional taxonomy that relies mainly on shell morphology has not been able to solve the problems involved with the phenotypic (sometimes ecophenotypic) variation of different clonal lineages that result from a mixture of such dovetailing phenomena as hermaphroditism, arogenetic reproduction, polyploidization, and introgressive hybridization (Kjiviriya et al., 1991; Komaru & Konishi, 1999; Pfenninger et al., 2002; Park et al., 2002; Lee et al., 2005).

All these effects render shell morphology alone, which is the principal information tapped from historical dry museum material (and so the types), not sufficient to resolve the phylogenetic relationships within the Corbiculidae. Particularly in the invasive species with clonal reproduction we will probably not be able to connect a certain morphotype with historical (type) specimens so that one can be confident that they also share the same either mitochondrial or nuclear genome. This problem certainly limits the value of historical specimens for modern genetic studies not only in corbiculids. However, the primary function of types as defined by the *International Code of Zoological Nomenclature* is not to fasciliate phylogenetic or population genetic studies but to "provide the objective standard of reference for the application of the name it bears" (ICZN 2000, Code Art. 61). In so far the importance of type material is not affected by their inability to serve genetic studies.

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A FIELD METHOD FOR QUANTIFYING THE GRAZING ACTIVITY OF SLUGS, WITH PARTICULAR REFERENCE TO *ARION LUSITANICUS* (MOLLUSCA)

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ABSTRACT

In earlier experiments soft, "plasticine" modelling clay was readily consumed by slugs. Therefore, a method for determining the grazing activity of *Arion lusitanicus* using plasticine baits was tested in the field. Plasticine was exposed on the surface of the soil in small trays shielded from mice by wire mesh screens. After several days of exposure, the quantity of plasticine consumed was determined by weighing. Laboratory experiments showed that the amount of plasticine consumed is proportional to slug body mass, plasticine is eaten over a wide range of temperatures between 6–25°C, and the presence of alternative vegetable food does not decrease consumption. In the field, consumption of such baits was positively correlated with slug numbers and revealed variation in slug grazing activity between sites during the course of a season. The use of plasticine baits is recommended for establishing slug feeding activity in the field. This is probably the best method available to date for quantitative studies. Plasticine was also eaten by several other species of slugs and snails.

Key words: baits, plasticine, consumption, temperature, food, grazing, activity, seasonal variation.

INTRODUCTION

Slugs are abundant on agricultural land in Central Europe and consume a variety of food items, including seedlings and mature plants. They become important crop pests, especially when the only plants available are cultivated (Cook et al., 1997; Glen & Moens, 2002; Moens & Glen, 2002). Even in mixed plant stands, they may have a significant effect and completely destroy populations of certain species, particularly seedlings. This is reported for several slug species and plant communities (Bruelheide & Scheidel, 1999; Frank, 2003; Buschmann et al., 2005). Therefore, the determination of slug abundance and activity is important and has been studied by many authors. There are several methods of determining slug abundance. In addition to direct counts and washing slugs from soil samples, various baits and traps are frequently used. That is, slug numbers may be determined, but obtaining precise estimates is laborious.

In a study of invertebrate seed predation (Honek et al., 2003), seeds were presented stuck into the surface of soft plasticine in small tin trays. In the field, the trays were placed in cages made of rectangular wire mesh (9 x 9 mm opening size) that prevented the access of mice, which will eat the plasticine. However, in some places, the plasticine was eaten by slugs to such extent that it was decided to replace soft plasticine by a kind of modelling clay that hardens on exposure to air (Honek et al., 2005). Even large slugs easily passed through wire mesh and were observed consuming the plasticine. The slug damage to the plasticine differed among sites. These difficulties, which made investigating seed consumption impracticable, lead us to the use of baits of soft plasticine to monitor slug grazing activity. In contrast to slug numbers, determining their grazing activity in the field remains unresolved. The consumption of baits of more natural food is not easy to quantify because of its quick deterioration. By contrast, the quantity of plasticine remains unchanged even after long exposure. We therefore tested consumption of this auspicious bait material under a variety of laboratory conditions and in the field.

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In our experiments, the grazing activity of *Arion lusitanicus* (Mabille) was investigated. This species was first established in the Czech Republic in 1991 and became widespread between 1996–2000 (Horsak & Dvorak, 2003). Arion lusitanicus is found in dense vegetation, swards, and hedges (Grimm & Paill, 2001). When population outbreaks occur, it colonizes the margins of crops and set-aside fields. It overwinters as an egg. Young animals are found from late April, mature in July–August, and die in October (Briner & Frank, 1998a; Grimm, 2001). In contrast to other slug species (Hommay et al., 1998), it is active during part of the day, until late morning and from early afternoon (Grimm et al., 2000), even on dry, warm and sunny days. *Arion lusitanicus* eats a variety of food, including living herbs, particularly seedlings and may change the species composition of wild herb stands. *Arion lusitanicus* is a serious pest of vegetables and garden plants (Fischer & Reischutz, 1998). The damage to field crops is occasional, limited to years of high abundance and to the margins of fields (Frank, 1998a, b).

This paper reports the results of using baits of soft plasticine for monitoring *A. lusitanicus* grazing activity. The experiments reveal the usefulness of this unnatural material for ecological studies. In particular, the results concerned (i) laboratory experiments to determine the factors that limit the consumption of plasticine and (ii) field experiments to determine the relationship between slug abundance and plasticine consumption, and its local, seasonal, and annual variation. Further laboratory experiments also (iii) determined plasticine consumption of other abundant species of molluscs.

MATERIALS AND METHODS

Presenting Plasticine

Plasticine modelling clay is a non-desiccating and non-solidifying malleable matter consisting of clay (frequently kaolin), oil (rape-seed, vaseline, zinc oleate), wax (bees or Japan), and minor components in various proportions (Barta, 1934). Plasticine used in all the experiments is manufactured by Jovi S. A. (Barcelona, Spain), which is commonly available in art shops. Its precise composition is not available. Pilot experiments have shown that this plasticine does not change in mass when exposed to conditions of 100% relative humidity and 25°C. Average difference after 24 h exposure was only + 0.2 ± 0.4% of the original mass. This plasticine was offered to slugs on tin trays (25 mm diameter, 5 mm deep) in the field, or on plastic trays (10 mm diameter, 3 mm deep) in the laboratory. The plasticine was pressed on to the trays so that its surface was convex. Light plastic trays were used in experiments involving single slugs, because weighing the amounts consumed was more precise. The trays were weighed before exposure to a 10^{-2} g accuracy. After presenting them to slugs, they were cleaned of adhering soil by a gentle flow of water, dried, and weighed. The difference in mass indicated the amount of slug grazing. Records were also made of consumption visually. In all experiments, the trays were replaced before they were completely empty so that the consumption of plasticine per unit time could be calculated.

Experimental Animals

Experiments on *A. lusitanicus* were made at Praha-Ruzyně (50°05'N, 14°15'E, altitude 340 m a.s.l.). Laboratory experiments were made using slugs collected at one place (dense uncut sward in an old pear garden). The slugs were collected randomly, put into a 0.5 l bottle, provided with leaves of herbaceous plants, sprayed with water, and stored at 5°C until the beginning of the experiment, always within 48 h.

Laboratory Experiments

The experimental arena was a plastic cup (8 cm diameter, 4.5 cm high). The bottom of each cup was covered with a 0.5 cm deep layer of field soil, which had passed through a 8 mm mesh sieve. The walls of the cup and the soil layer were sprayed with sufficient tap water for the soil not to become muddy. Plastic trays with plasticine were then placed into the center of each arena (Fig. 1). The experimental slugs were weighed to an 10^{-2} g accuracy, and one was put randomly into each of the experimental arenas. The cups were covered with a perforated plastic lid. The experimental animals were maintained at 16 h light : 8 h dark photoperiod and 25°C (unless indicated otherwise). The experiments started between 16:00–18:00 h, and lasted for 24 h (unless indicated otherwise). The slugs were then removed and the weight of plasticine consumed determined.

The determination of the relationship between slug body mass and plasticine consumption was carried out on June 8, 2005 (N = 25 individuals) and repeated on July 7, 2005 (N = 20). The experiment establishing the consumption of plasticine over ten days was made on July 19–29, 2005 (N = 20), and consumption was established twice, on July 24 (when the trays were replaced) and on July 29. The effect of alternative food was determined on July 20, 2005. The slugs were provided either with plasticine plus a piece of slug (*A. lusitanicus*) carcass (N = 15), or with plasticine plus a fresh leaf of each of seven broadleaved plants: *Aegopodium podagraria* L., *Bellis perennis* L., *Crepis biennis* L., *Geranium pratense* L., *Plantago major* L., *Taraxacum officinale* Weber ex Wiggers, and *Urtica dioica* L. (N = 15). All species of plants were common at the place where the slugs were collected. Intact leaves of these plants were collected just before the start of the experiment. The effects of temperature on consumption of plasticine was determined on September 6, 2005 (N = 12 at each temperature). The slugs were kept in climatised boxes at temperatures of 3, 6, 10, 15, 20, 25, or 30°C, which varied by ± 0.5°C. The dates are given because the experiments revealed that grazing activity may vary with the course of the season.

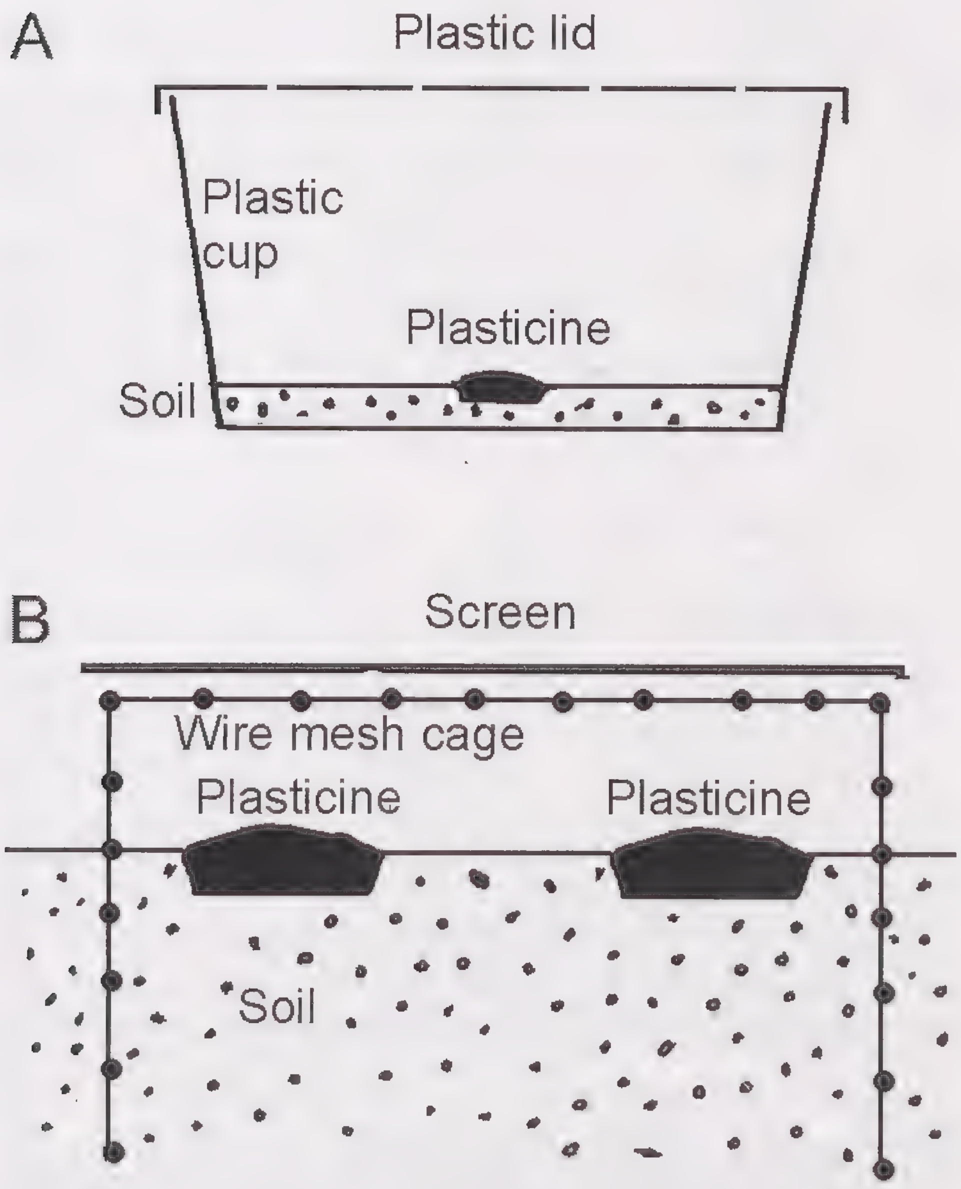

FIG. 1. Methods of exposing plasticine to slug grazing. A: In the laboratory; B: In the field. See Materials and Methods for detailed descriptions.

Field Experiments

Field experiments were made within the experimental area of the Research Institute of Crop Production at several sites, which differed in *A. lusitanicus* abundance. This large species was the dominant consumer of plasticine, because other large slugs (*Limax maximus* L., *Arion hortensis* s.l.) were rare, and small slugs (*Deroceras* sp.) only ate negligible amounts of plasticine. At each site, two tin trays filled with plasticine were placed in a 15 x 15 cm plot from which vegetation was removed and the soil loosened to a depth of approximately 10 cm. The trays were let into the soil so that plasticine surface was level with the soil surface. They were immediately covered with a roof-like cage of 10 x 10 cm area with 6 cm side walls, let into the soil to a depth of 4 cm. The cages were of 10 mm square mesh made of 1 mm thick wire (Fig. 1), which allowed access to slugs but prevented rodents from eating the plasticine.

In experiment (i) the relationship between slug abundance and plasticine consumption was established. On July 7, 2005, between 18:30–20:00 h, slugs were counted at 12 sites. At each site, slug numbers were established on ten plots of 0.25 m^2 size, randomly placed within an 6 x 6 m area centered around the place where the plasticine baits were placed. The slugs were counted by manually spreading the vegetation and counting all the visible individuals. Plasticine baits were put out on July 8–12, 2005. At each site, four cages were placed in the corners of a 1 x 1 m quadrat. In experiment (ii) the correlation between plasticine grazing activity in two successive periods, July 27–August 26, 2004, and August 26–September 17, 2004, was established. The cages were placed at eight sites, in the same arrangement as in experiment (i). In 2004, slugs were uncommon and grazing activity was so low that the plasticine did not have to be replaced within each of the experimental

TABLE 1. Consumption of plasticine by molluscs. Species, date of collection (2005), and consumption of plasticine (n = number of individuals that did (Yes) or did not (No) eat the plasticine.

Species	Date	n	Consumption	
			Yes	No
Agriolimacidae				
Deroceras agreste (Linnaeus, 1758)	12-July	7	7	
Deroceras reticulatum (O. F. Müller, 1774)	*	9	9	
Arionidae				
Arion circumscriptus Johnston, 1828	24-July	4	3	1
Arion hortensis s.l.	8-July	6	6	
Bradybaenidae				
Fruticicola fruticum (O. F. Müller, 1774)	14-July	9	9	
Clausiliidae				
Balea biplicata (Montagu, 1803)	26-July	9	2	7
Cochlodina laminata (Montagu, 1803)	26-July	27	27	
Macrogastra ventricosa (Draparnaud, 1801)	16-July	2		2
Cochlicopidae				
Cochlicopa lubrica (O. F. Müller, 1774)	26-July	11		11
Discidae				
Discus ruderatus (A. Férussac, 1821)	16-July	11	11	
Enidae				
Chondrula tridens (O. F. Müller, 1774)	8-July	3		3
Zebrina detrita (O. F. Müller, 1774)	14-July	1		1
Helicidae				
Arianta arbustorum (Linnaeus, 1758)	10-July	9	9	
Cepaea hortensis (O. F. Müller, 1774)	8-July	12	11	1
Cepaea nemoralis (Linnaeus, 1758)	8-July	6	6	
Cepaea vindobonensis (A. Férussac, 1821)	14-July	5	4	1
Helicigona lapicida (Linnaeus, 1758)	10-July	1	1	
Helix pomatia Linnaeus, 1758	5-July	8	7	1
Hygromiidae				
Monachoides incarnatus (O. F. Müller, 1774)	26-July	11	5	6
Trichia hispida (Linnaeus, 1758)	14-July	2		2
Urticicola umbrosus (Pfeiffer, 1828)	26-July	38	11	27
Xerolenta obvia (Menke, 1828)	7-July	6	6	
Limacidae				
Lehmania marginata (O. F. Müller, 1774)	16-July	5	1	4
Limax cinereoniger Wolf, 1803	16-July	2	2	
Limax maximus (Linnaeus, 1758)	26-July	4	4	
Malacolimax tenellus (O. F. Müller, 1774)	24-July	4	4	
Milacidae				
Tandonia rustica (Millet, 1843)	24-July	1	1	
Succineidae				
Succinea putris (Linnaeus, 1758)	26-July	8	7	1
Zonitidae				
Oxychilus cellarius (O. F. Müller, 1774)	26-July	8	2	6

periods. Experiment (iii) was done in 2003–2005. Each year, two cages were put out at a grassy site (high abundance of slugs) and a set-aside field (low abundance), from April 10 until October 31. The distance between the sites was 70 m. The trays were replaced weekly and at the time of maximum grazing activity in 2005, twice a week.

Grazing by Other Slugs and Snails

Plasticine was offered to another 29 common species of slugs and snails collected at several localities in western Czech Republic, between July 8–26, 2005 (Table 1). Species were identified according to Lozek (1956) and Kerney & Cameron (1999). If possible ≥ 5 individuals of each species was tested, except for rare species. They were collected in 0.5 l plastic bottles and within 24 h offered plasticine under the same conditions as in the laboratory experiments using *A. lusitanicus* (25°C, 16 h light : 8 h dark photoperiod). Plasticine was offered for one day except for those animals that did not readily eat the plasticine, when the period of exposure was extended to one week. The animals that scraped the surface of the plasticine were classified as "eating plasticine", those that did not as "not eating plasticine". If the molluscs were small and the presence of scrapes dubious, each individual was kept for 24 h in a 9 x 56 mm glass tube and its faeces examined. Eating plasticine resulted in the production of white faeces.

Data Processing

For the laboratory results, the correlation and linear regression of plasticine consumption on slug fresh body mass were calculated and the differences between slopes and intercepts of regression lines tested using Generalised Linear Models in R system, a free statistical product available at the Internet (R Development Core Team, 2004). The weight of plasticine consumed was standardized per unit *A. lusitanicus* mass. The differences in standardised consumption were tested using one-way ANOVA, with consumption in particular replicates as a response variable and treatment (slug age, alternative food, temperature) as factors. The differences between numbers of slugs eating plasticine at different temperatures were tested by chi-sqaure test. In the field, average plasticine consumption tray^{-1} over the experiment period was calculated for particular experiment sites (experiments i and ii). Consumption was regressed on slug numbers m^{-2} (experiment i), or consumption during one period was regressed on that in the other period (experiment ii). In experiment (iii), mean consumption day^{-1} trap^{-1} was calculated for each week or shorter period of exposure and plotted against time. Means (± SE) are given throughout the paper.

RESULTS

Laboratory Experiments

Plasticine consumption increased significantly ($p < 0.001$) and proportionately with the body mass of the slug, more so on June 8 than July 7 (Fig. 2). Average standardised consumption was also significantly ($p < 0.001$) higher earlier (0.082 ± 0.004 g . g slug body mass^{-1} . day^{-1}) than later in the year (0.039 ± 0.003 g . g slug body mass^{-1}. day^{-1}). Standardised consumption decreased as average slug body mass increased, from 1.53 ± 0.133 g on June 8 to 3.44 ± 0.445 g on July 7. Plasticine was consumed by all the slugs.

In the ten day no-choice experiment, plasticine was consumed by all experimental animals (n = 20), more on days 1–5 (0.012 ± 0.001 g . g slug body mass^{-1} . day^{-1}) than days 6–10 (0.005 ± 0.001 g . g slug body mass^{-1} . day^{-1}) of the experiment. The slugs thus gradually ceased eating plasticine, which they originally eagerly consumed. No slugs died during the ten-day experimental period, but they lost an average of 15.3 ± 2.0% of their original mass.

Providing alternative food influenced plasticine consumption. Only four of 15 experimental slugs (27%) provided with *A. lusitanicus* carcasses ate plasticine, and their consumption of plasticine (0.014 ± 0.006 g . g slug body mass^{-1} . day^{-1}) was significantly less (ANOVA: $F_{1,17} = 6.694$, $p < 0.05$) than that of slugs offered leaves. All the experimental slugs eagerly consumed conspecific carcasses.When provided with leaves of seven broadleaved

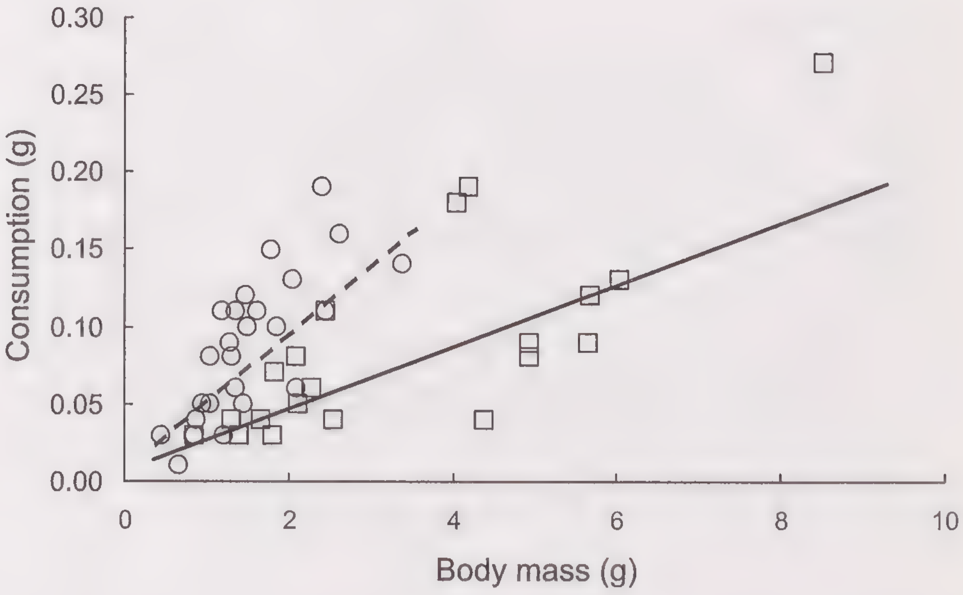

FIG. 2. The quantity of plasticine consumed vs. wet body mass of *A. lusitanicus*, established on June 8 (o , n = 25, a = 0.0517, b = 0.0084, $F_{1,23} = 30.64$, $p < 0.001$) and July 7 (, n = 20, a = 0.0238, b = 0.0067, $F_{1,18} = 24.65$, $p < 0.001$).

herbaceous plants, all experimental slugs (n = 15) ate plasticine (0.035 ± 0.004 g . g slug body mass^{-1} . day^{-1}). Plasticine was consumed despite herbivory: fourteen slugs nibbled at least one leaf. They preferred the leaves *A. podagraria* and *P. major* (both eaten by six individuals), *C. biennis* (five), followed by *T. officinale* (three) and *B. perennis* (two). Leaves of *G. pratense* and *U. dioica* were avoided. Plasticine consumption was similar to that recorded in the no-choice experiment of July 7, although the experimental slugs were heavier (4.62 ± 0.420 g).

Plasticine was eaten over a wide range of temperatures between 6–25°C (Fig. 3). The optimum temperature was 15°C, at which the proportion of slugs eating plasticine and the quantity eaten per individual was highest. The consumption decreased below and above 15°C. The numbers of slugs eating plasticine differed significantly (chi-square = 18.81, $p < 0.005$), but the standardised consumptions did not differ significantly (ANOVA: $F_{4,32} = 0.794$, NS). No consumption of plasticine was recorded at the lowest (3°C) and the highest (30°C) temperatures, probably because the change in slug behaviour.

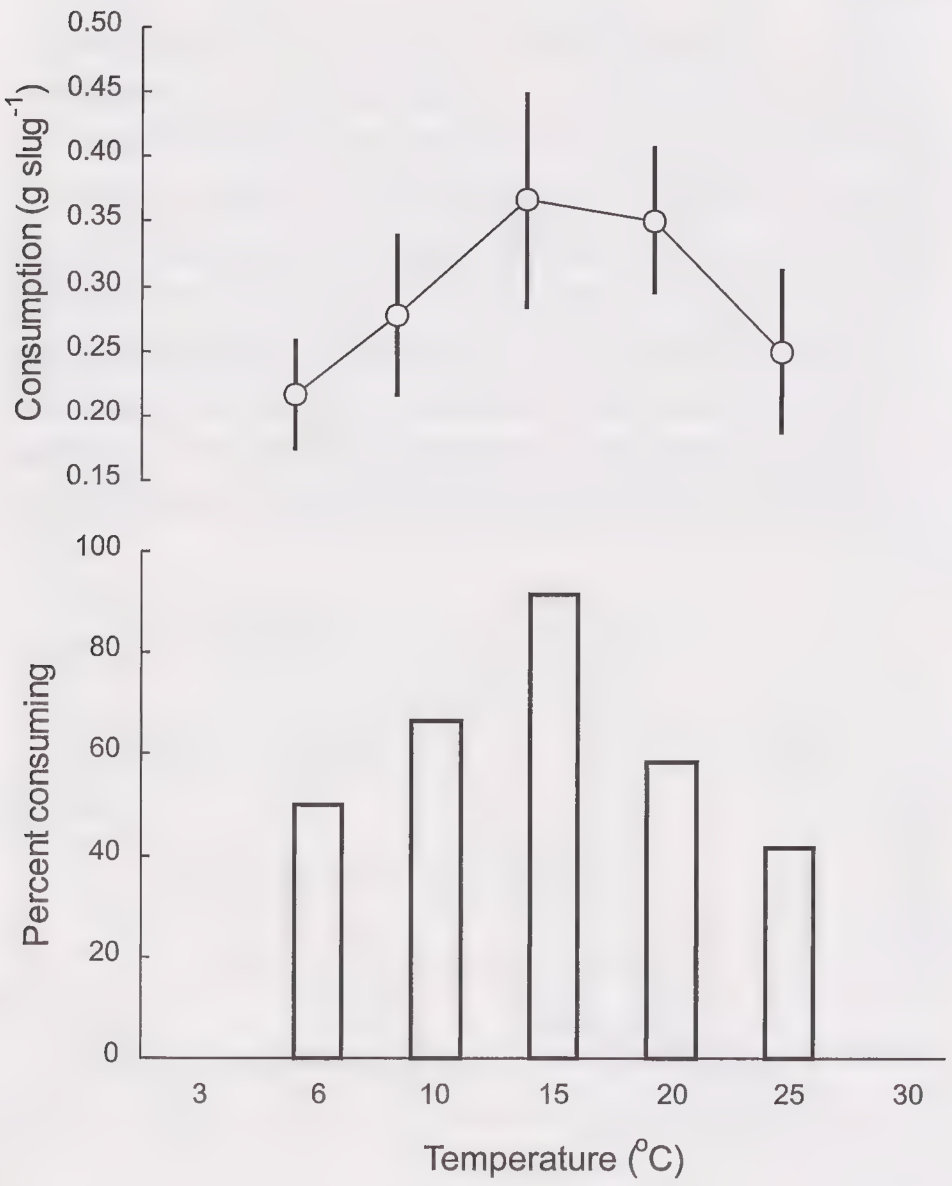

FIG. 3. The effect of different constant temperatures on plasticine consumption, September 6, 2005. Above: mean consumption slug^{-1} ± SE (calculated only for individuals that ate plasticine). Below: percentage of slugs eating plasticine (N = 12 at all temperatures). Plasticine was not eaten at 3 and 30°C.

Field Experiments

The average quantity of plasticine consumed over the four-day period (0.0–4.1 g tray^{-1}) differed significantly between the 12 sites (ANOVA: $F_{11,36} = 31.43$, $p < 0.001$) and significantly depended on slug abundance (Fig. 4). Average plasticine consumption at eight other sites was significantly correlated ($R^2 = 0.505$, $p < 0.05$) in the two successive periods, with a total duration was 52 days (Fig. 5). This revealed that the mosaic pattern of grazing activity persisted in time, which suggests that patterns of slug abundance are maintained through a season.

Seasonal variation in plasticine consumption was similar in 2003–2005 (Fig. 6). Grazing activity was in all years maximum in July and August and lower before and after this period. At the grassy site, peak consumption was approximately ten times greater in the year of high slug abundance (2005) than in previous years, when slug abundance was low. At the other site (set-aside field), the annual differences in plasticine grazing were similar, although the maximum consumption was approximately ten times lower than at the grassy site. There was virtually no feeding on plasticine in 2003 and 2004, while some consumption occurred in 2005, when some *A. lusitanicus* colonized the fallow from more favourable surrounding sites.

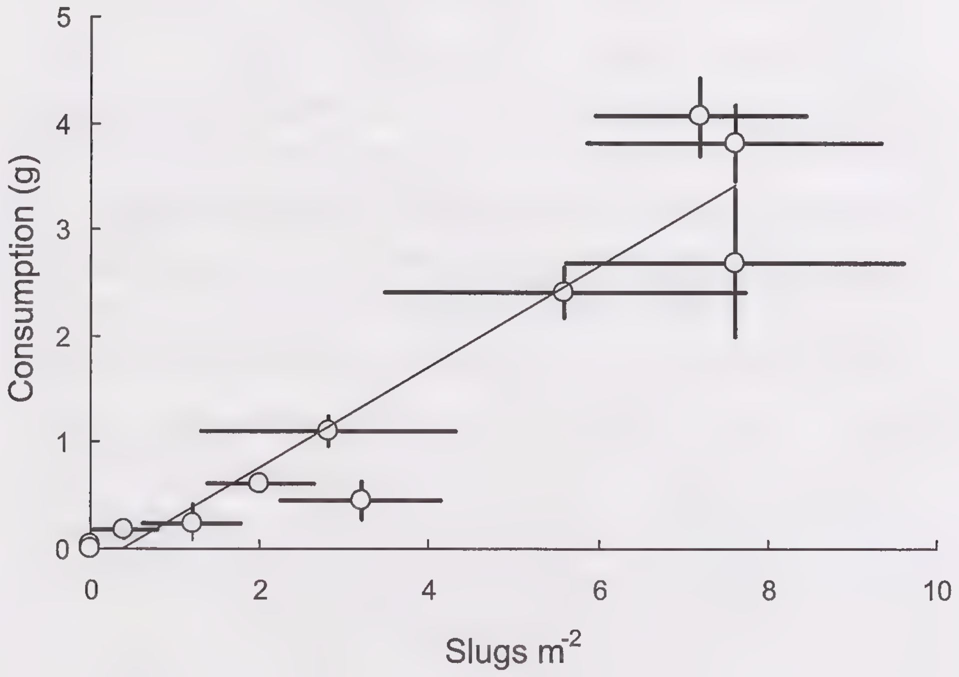

FIG. 4. Average plasticine consumption ± SE (g tray^{-1} eaten on July 8–12, 2005) vs. average numbers m^{-2} ± SE of *A. lusitanicus* (July 7, 2005), at 12 sites at Praha-Ruzyne (a = 0.4722, b = -0.1767, $F_{1,10} = 99.70$, $p < 0.001$).

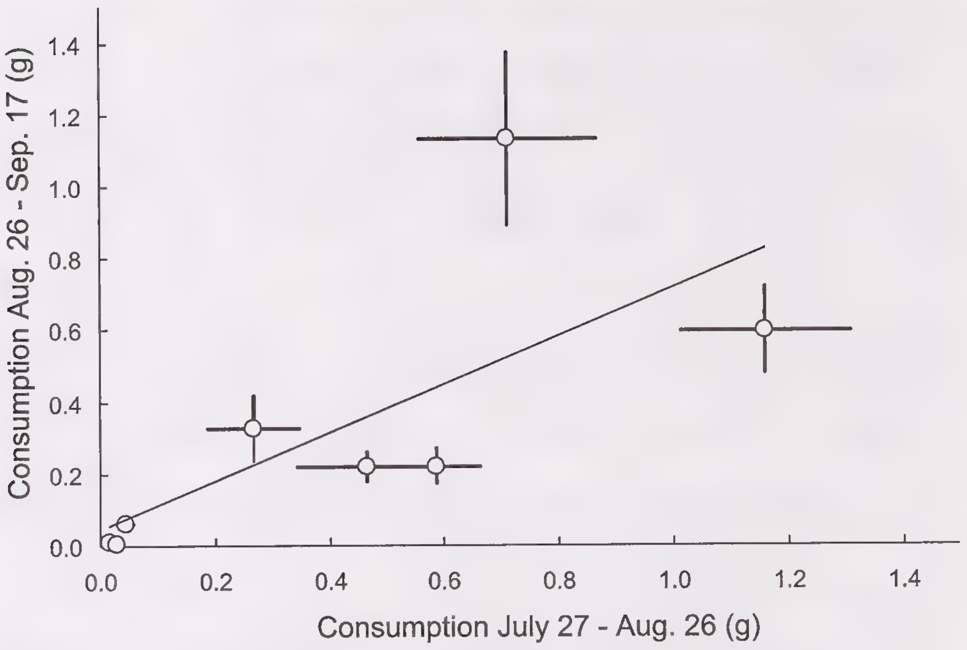

FIG. 5. Average plasticine consumption ± SE (g tray^{-1} period^{-1}) at eight sites at Praha-Ruzyne on Aug. 26–Sep. 17, 2004 vs. consumption on the same sites on July 27–Aug. 26, 2004 ($a = 0.6716$, $b = 0.0467$, $R^2 = 0.5053$, $p < 0.05$).

Grazing by Other Slugs and Snails

Laboratory testing of 29 species of slugs and snails (Table 1) revealed that *A. lusitanicus* is not the only species that eats plasticine. It was readily eaten by large snail species of the families Bradybaenidae and Helicidae, and all species of slug tested, except *L. marginata.* Mixed responses were obtained in the family Hygromiidae, in which *X. obvia* readily accepted plasticine, whereas other species were reluctant to eat it, or some individuals did and others did not eat. Few species were reluctant to eat plasticine, mostly small (*C. lubrica*, *O. cellarius*) and medium sized (*C. tridens*, *Z. detrita*) snails.

DISCUSSION

The results revealed that determining slug grazing by means of plasticine baits is a useful tool for establishing the grazing activity and abundance of *A. lusitanicus*. Laboratory experiments revealed the limitations of this method. The most important factor decreasing plasticine consumption was the presence of alternative food. Because of marked cannibalism in *A. lusitanicus*, dead conspecifics were more attractive than plasticine baits and significantly decreased the consumption of plasticine. Many *A. lusitanicus* are often killed on roads by traffic, and near such "carnage" feeding on plasticine may be confounded by cannibalism. Moreover, *A. lusitanicus* is attracted by baits of vertebrate meat (Z. Martinkova, unpubl.) and presence of an animal carcass may have the same local effect as dead slugs. By contrast, although *A. lusitanicus* eat many species of plants (Briner & Frank, 1998b; Keller et al., 1999), the presence of preferred species, for example, *A. podagraria* (Kozlowski & Kozlowska, 2000), did not reduce the consumption of plasticine.

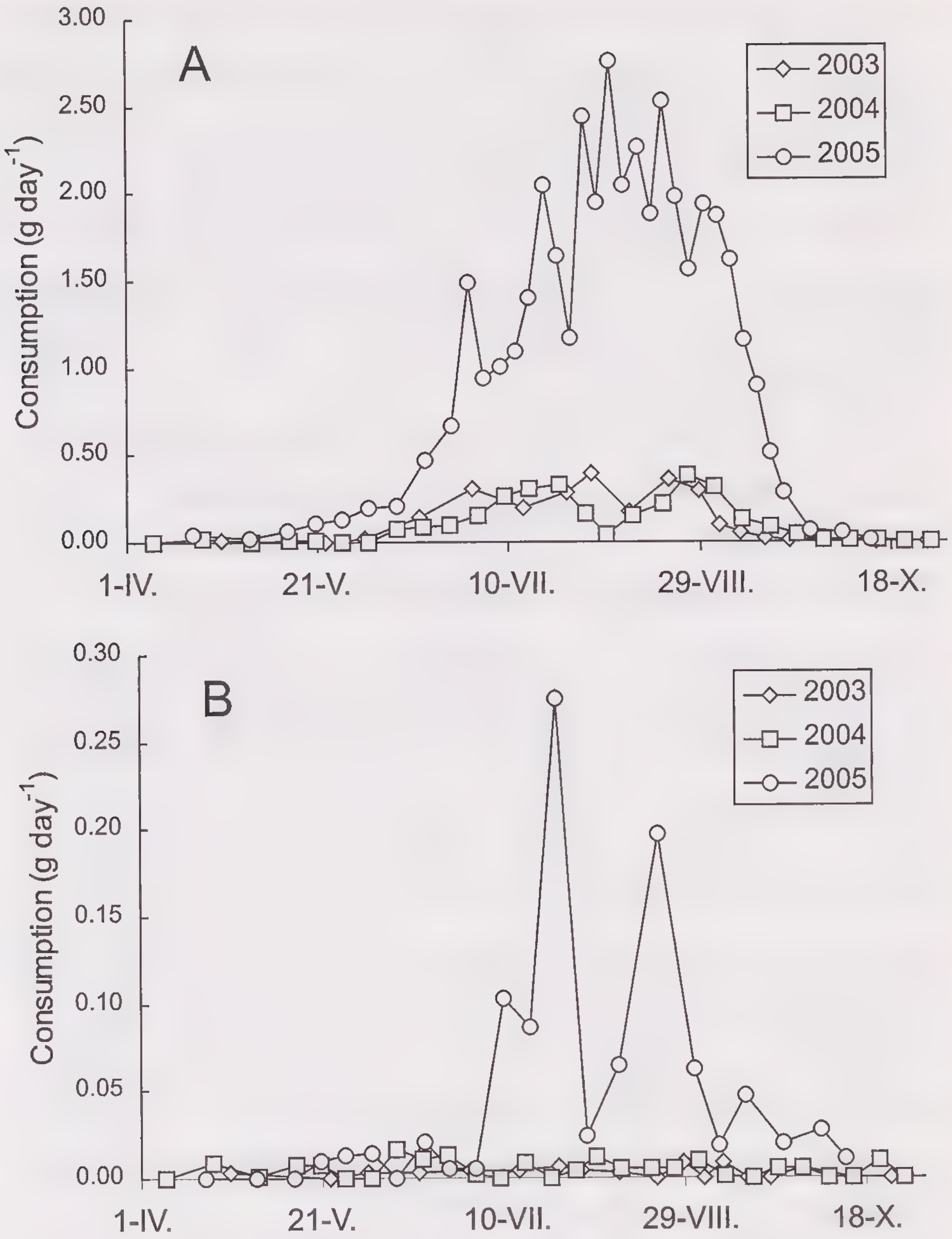

FIG. 6. Consumption of plasticine (g tray^{-1} day^{-1}) through the vegetative season (April–October) 2003–2005. A: Grassy plot; B: Set-aside field, at Praha-Ruzyne. Note different scales on ordinates.

Plasticine was eaten over a wide range of temperatures, and feeding ceased only at low (3°C) and high (30°C) temperatures. At intermediate temperatures, there was a variation in the proportion of individuals eating plasticine and in the amount consumed per individual, with a maximum at 15°C. In our experiment, this variation was probably apparent because consumption was measured late in the season, on September 6, when feeding activity of field collected animals was low (Fig. 6). In June–August, when it was at a maximum, slugs might be less affected by differences in temperature. This was apparent in the laboratory experiments made in June and July, at room temperature of 25°C, when slugs feed the plasticine well. Tolerance of a wide range of temperatures is probably an essential feature of *A. lusitanicus* and enables this species to remain active over a large part of the day, except for the warmest period in the early afternoon (Grimm et al., 2000). Quality of plasticine (viscosity and/or release of volatiles) also might have change at the upper and lower temperatures.

The experiments in the field revealed spatial variation in *A. lusitanicus* grazing. This local variation in plasticine consumption persisted within a season but may differ between years. The changes were due to annual variations in overall slug abundance that resulted in some places only being colonized in years when slugs were abundant. The changes may be inferred from reports of pest occurrences involving *A. lusitanicus* registered by the national Phytomedicine Survey. They were uncommon in 2003 and 2004, but increased sharply in abundance in 2005 (J. Rehak, personal communication). In our experiments, this change resulted in, for example, a change in grazing activity revealed in the set-aside field, where there was no grazing in 2003 and 2004, but plasticine was consumed in 2005. Direct observation of the plasticine baits revealed that other slug species, particularly *D. reticulatum*, contributed to grazing activity late in the season (October–November), when *A. lusitanicus* was already absent. However, because grazing activity at this period was low compared to July–August (Fig. 6), the contribution of species other than *A. lusitanicus* to overall grazing was very small.

Determining slug numbers from plasticine grazing appears at first sight to be difficult. Although consumption of plasticine in the laboratory was proportional to slug mass, the relationship was weak and the consumption per unit body mass changed during the course of the season. Moreover, the small home ranges of *A. lusitanicus* (Grimm & Paill, 2001) might prevent random access of individuals to plasticine baits. Despite these confounding factors, plasticine grazing was a good indicator of *A. lusitanicus* numbers at particular sites.

What are the advantages of plasticine baits compared to the other methods of establishing the presence of slugs in the field? The most frequently used methods are direct sampling (Frank, 1998d), establishing enclosures (Theenhaus & Schaefer, 1999), flooding samples slowly submerging them in water (Bohan et al., 2000), washing snails over sieves (Iglesias et al., 2001), seting traps of cardboard (Grimm & Paill, 2001) or other materials (Hanley et al., 1996; Scheidel & Bruelheide, 2001), and exposing baits consisting of preferred food (Frank, 1998c). These methods can be calibrated to reveal slug abundance but not grazing activity. The use of plasticine bait in the field thus appears to be a convenient new method for establishing slug grazing activity and has already proved useful in studies of weed seedling mortalitiy due to *A. lusitanicus* (A. Honek, Z. Martinkova & P. Saska unpubl.).

Plasticine baits might be useful also for studies on the grazing activity of other slugs. For small species of Agriolimacidae, however, the method of exposure in the field should be modified, because they consume very little, which is difficult to establish by weighing. Feeding marks on the surface of the plasticine probably could be evaluated optically. However, the method cannot be used for investigating snail activity, perhaps with the exception of small species, like *X. obvia*. This is because plasticine is readily consumed by mice and therefore should be surrounded by cages of wire mesh with maximum approximately 1 cm wide openings. Our observations indicate (A. Honek & Z. Martinkova unpubl.) that this mesh size permits access of even the largest specimens of *A. lusitanicus*, approximately 12 cm long. However, access of large snails is prevented because of their shell size.

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EMPIRICAL ESTIMATES OF REPRODUCTIVE ISOLATION AMONG THE FRESHWATER PULMONATE SNAILS *PHYSA ACUTA*, *P. POMILIA*, AND *P. HENDERSONI*

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ABSTRACT

Physa hendersoni collected from its type locality near Yemassee, South Carolina, and *Physa pomilia* from its type locality near Claiborne, Alabama, both display the penial morphology that has been characterized as "type-bc" by Te (1978, 1980). Mate choice tests returned no evidence of premating reproductive isolation between these two populations, and no-choice breeding experiments confirmed outcross fecundity, F1 viability and F1 fertility comparable to incross controls. Significant premating reproductive isolation was documented, however, between the *P. hendersoni* population and a population of *Physa acuta* from Charleston, South Carolina, bearing the "type-c" penial morphology. No-choice breeding experiments involving *P. acuta* and *P. hendersoni* yielded a mixture of hybrid and selfed progeny, the hybrids apparently sterile. Thus the nomen *Physa hendersoni* is a junior synonym of *P. pomilia*, whereas *P. pomilia* and *P. acuta* are distinct biological species.

Key words: Gastropoda, Pulmonata, *Physella*, speciation, mate choice, allozyme electrophoresis.

INTRODUCTION

The most influential classification system for the Physidae at present is that of George Te (1978, 1980; Burch, 1989). Te recognized approximately 40 species and subspecies of physids in North America, divided into genera and subgenera by penial morphology. Experimental breeding studies have subsequently suggested, however, that Te's estimate of specific diversity may have been too high. Among the nominal species bearing the penial morphology Te characterized as "type-c", *Physa cubensis*, *P. heterostropha*, *P. integra*, and *P. virgata* have all recently been synonymized under *P. acuta*, described from France prior to any American physid (Dillon et al., 2002, 2005a; Paraense & Pointier, 2003). Among the nominal species bearing Te's "penial complex type-b," recent research has suggested that *P. ancillaria*, *P. aurea*, *P. microstriata*, *P. parkeri*, *P. sayii* and *P. utahensis* may all be junior synonyms of *P. gyrina* (Dillon & Wethington, 2006a, b). Reproductive isolation seems to be complete, however, between physids bearing type-b and type-c penial complexes (Dillon et al., 2004).

No attention has yet been directed, however, toward reproductive relationships in physids bearing the penial complex characterized by Te (1978) as "type-bc," intermediate between the two more common morphologies discussed above. Te attributed the type-bc penis to a set of four species inhabiting the American South, including *Physa hendersoni*, originally described by Clench (1925) as a subspecies of *P. pomilia* (Conrad, 1834). Te's observations suggested to him that *P. pomilia* had a type-c penis, however, which led him to propose that *P. pomilia* be considered a subspecies of the widespread *P. heterostropha*, which has subsequently been synonymized under *P. acuta*. Te raised *P. hendersoni* to the full species level, listed its range as extending from "West Virginia, Tennessee, and Missouri south to the Carolinas, Mississippi, and Florida", and recognized several subspecies within it (Burch, 1989).

Wethington (2003) has reported, however, that specimens freshly collected from the type locality of *Physa pomilia* bear a type-bc penial complex, not type-c as suggested by Te. Molecular data further demonstrate a close genetic similarity between *P. hendersoni* and *P.*

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pomilia, as originally proposed by Clench, and do not suggest an especially close affinity between *P. pomilia* and *P. acuta* (Wethington & Lydeard, in press). The purpose of the present work is to use experimental methods to test for reproductive isolation between three populations of physids – *Physa hendersoni*, *P. pomilia*, and *P. acuta* – the first two bearing type-bc penial morphology, the third a type-c physid, with which *P. pomilia* has been confused.

METHODS

The type locality given by Draparnaud (1805) for *Physa acuta* was the "River Garonne," that given by Conrad (1834) for *P. pomilia* was "Randon's Creek, near Claiborne, Alabama," and that given for *P. pomilia hendersoni* by Clench (1925) was "Yemassee, Beaufort Co., South Carolina." Our line "H" was established from snails collected in the Combahee River at the US 21/17A bridge, 1 km E of Yemassee, Hampton County, South Carolina (32.7060°N, 80.8281°W). Line "P" was founded from snails collected in Randons Creek at the CR 23 bridge, 12 km S of Claiborne, Monroe County, Alabama (31.4387°N, 87.5445°W). Our control line of *Physa acuta* ("A") was established from snails collected in the main pond at Charles Towne Landing State Park, west of the Ashley River, within the city limits of Charleston, South Carolina (32.8062°N, 79.9862°W). Snails of this population are not reproductively isolated from near-topotypic *P. acuta* populations sampled from France (Dillon et al., 2002). The Charleston population has previously been designated "Ctl" by Dillon & Wethington (1995), "A" by Dillon et al. (2004), and "C" by Dillon et al. (2005a). The habitat has been described by Dillon & Dutra-Clark (1992).

Wild-collected adult snails were returned to the laboratory and isolated in 10 oz. (210 ml) clear polyethylene drinking cups of aerated, filtered pond water with Petri dish covers, and fed a commercial *Spirulina*-based flake fish food, finely ground. All culture took place at approximately 23°C in a 12:12 light cycle. Every two or three days, each snail was transferred to a fresh cup, with feeding and water change, leaving egg masses attached to the walls of the previous cup. The hatchlings from these egg masses, conceived in the wild but laboratory born, served as experimental animals both for our mate choice tests and as the parental generation for our no-choice tests for reproductive isolation.

We performed two separate mate-choice experiments to assess prezygotic reproductive isolation, one comparing populations A and H, and a second comparing populations H and P. Each experiment involved 30 adult snails per population. Thus, we reared to maturity 30 snails from lines P and A, and 60 snails from line H, isolated in individual cups with weekly feeding and water change. The experiments (designated AH and HP) were both composed of three trials, involving ten snails from each population per trial, similar in shell size, marked with tiny spots of contrasting nail varnish. The 20 snails were introduced simultaneously into a two-liter glass beaker containing 1,400 ml of filtered pond water, placed on a glass countertop to facilitate observation on all sides, including the bottom.

Each trial was monitored for six hours. When a copulation was observed, defined as the complete insertion of the penis of one partner into the mantle cavity of a second, both snails were removed and their lines of origin noted. Then the shell of the individual serving as male was marked with a dot of white correction fluid, and both snails returned to the beaker. Only the first mate choice of each snail copulating as a male was recorded. Subsequent copulations undertaken by an individual already marked with white fluid were allowed to proceed undisturbed. Thus, the maximum number of data recorded for each trial was 20, although most trials concluded before all 20 individuals had copulated in the male role. Observations were combined across the three trials for each of the two experiments, AH and HP, and tested for evidence of sexual isolation with a chi-square statistic, normalized by 4/N (Gilbert & Starmer, 1985).

We also performed two separate no-choice experiments for postzygotic reproductive isolation, one between lines A and H, and the second between lines H and P. Each experiment involved the offspring of ten different wild-collected snails, randomly chosen at approximately 2 mm shell length, well before maturity. These juveniles (the parental generation) were paired in cups with individuals of a second population, for example A1 x H1, A2 x H2, ..., A10 x H10 and H1 x P1, H2 x P2, ..., H10 x P10. Two sets of incross controls were established simultaneously with each set of outcrosses, for example A1 x A2, A2 x A3, ..., A9 x A10, A10 x A1, and H1 x H2, H2 x H3, ..., H10 x H1. Thus, both experiments were initiated with 30 pairs of unrelated parental snails: 10 outcrosses and 20 incross controls.

All parental pairs were fed each week, and their water changed. When egg masses were observed, the embryos were counted and the pair of adults advanced to a fresh cup. The viable, crawling F1 hatchlings in each cup were enumerated two weeks subsequently. Observations on any pair of snails were terminated upon the death of either partner. Experiments proceeded for ten weeks beyond the week that a minimum of three pairs of parents first laid viable embryos, determined separately for the outcross and its two corresponding incross sets.

Only a small subset of the F1 progeny were retained and reared – the offspring of three unrelated pairs of parents per set, for example A1 x H1, A2 x H2 and A3 x H3 or A1 x A2, A3 x A4, and A5 x A6. Because both the AH experiment and the HP experiment involved an outcross and two incrosses, a total of 2 x 3 x 3 = 18 F1 sibships were retained, each sibship composed of up to ten cups of graded age. Randomly selected 2 mm individuals were paired between F1 sibships within sets, for example, AH1 x AH2, AH2 x AH3, AH3 x AH1. These F1 x F1 crosses were performed three times, as the F1 generation aged. So there were nine pairs of F1 animals for each set – three early, three middle, and three late – for a total of 9 x 3 = 27 such pairs for the AH experiment and 27 pairs for the HP experiment. These were reared to adulthood, with the week at first production of viable F2 hatchlings recorded.

At least 30 additional F1 progeny from the AH and HP outcrosses were reared to adulthood and their hybrid status confirmed via allozyme electrophoresis. We have previously identified 12 allozyme-encoding loci at which *Physa* populations commonly display polymorphisms interpretable as the product of codominant alleles, segregating in Mendelian fashion (Dillon & Wethington, 1994, 1995, 2006b; Wethington & Dillon, 1991). These are aconitase (ACON), esterases (EST, 3 loci), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (ISDH, 2 loci), leucine aminopeptidase (LAP), mannose phosphate isomerase (MPI), 6-phosphogluconate dehydrogenase (6PGD), and phosphoglucomutase (PGM, 2 loci). Detailed electrophoretic methods are available in Dillon (1992).

Evidence for reproductive isolation was assessed by comparing the fitness of the outcross pairs to the incross controls using five statistics: the age at first parental oviposition, the average weekly production of embryos by parents, the proportion of the embryos hatching into viable F1 juveniles, the proportion of the F1 pairs producing F2 embryos, and the age at which the first viable F2 progeny hatched. Significant depression demonstrated by the outcross below both of the controls in any of these broad measures of fitness would constitute evidence of postzygotic reproductive isolation.

Any difference in the central tendency of age at first oviposition between the ten outcross pairs and either set of ten incross controls was tested by calculating a combined (20-pair) median and comparing counts above and below that median using Fisher's exact tests.

The embryos laid by each pair of snails were summed, then divided by the total weeks of record (ignoring prematurity zeros and postmortem zeros) to derive an average weekly fecundity for each pair of parents. Then the central tendency in average weekly fecundity across the ten outcross pairs was compared to the two corresponding incross controls using Kruskal-Wallis nonparametric analysis of variance, with two degrees of freedom. *Post hoc* analysis, if appropriate, was performed with a set of Wilcoxon rank sum tests. The statistical analysis of F1 viability was similar to that for parental fecundity. The total viable hatchlings produced by each pair of parents was divided by their total embryos laid to obtain an overall percent hatching success for each pair. Then the central tendencies in hatching success across the ten outcross pairs and the two sets of ten incross controls were tested for significant differences using Kruskal-Wallis nonparametric ANOVA, with Wilcoxon *post hoc* tests if appropriate.

Differences in the central tendency of the date at which the first viable F2 progeny hatched were tested using statistical methods analogous to those described above for age at first reproduction in the parental generation. In this case, the week at which each of the (typically 9) F1 x F1 pairs yielded their first viable, crawling juveniles was recorded, a combined (18 pair) median calculated across both of the sets to be compared, and any difference in the number of observations above and below that median assessed using Fisher's exact tests.

RESULTS

Our AH mate choice tests revealed significant premating reproductive isolation between *Physa hendersoni* collected from its type locality and control *P. acuta* collected from Charleston (Table 1, upper). The effect appeared mutual, snails of line A over three times more likely to mate as males with other line A snails, and H snails more than twice as likely to mate as males with other H animals. Only 41 copulations were observed in the 6 hours allotted (of 60 possible), 30 of which were homogametic (normalized $\chi^2 = 6.86$, $p = 0.009$). The complex interactions among non-reciprocally mating simultaneous hermaphrodites have been described by Wethington & Dillon (1996).

Mating was much more frequent in the HP mate choice tests, with 57 (of 60) snails ultimately mating as male (Table 1, lower). No evidence was detected of reproductive isolation between *P. hendersoni* and *P. pomilia*, however, lines H and P apparently mating randomly (normalized $\chi^2 = 0.74$, $p = 0.39$).

Reared together in a no-choice design, *Physa acuta / P. hendersoni* pairs demonstrated slight, nonsignificant delays in age at first reproduction (p = 0.179, Table 2). The H controls tended to initiate oviposition at an earlier age and die at an earlier age under our experimental conditions, only four control pairs and two outcross pairs surviving the entire 10-week experiment (Fig. 1). Mean weekly parental fecundity was lower in the AH outcross than in the controls, although again this was not significant (p = 0.08), nor was any significant difference detected in F1 viability (p = 0.76). Fertility was, however, strikingly reduced in the F1 generation reared from the AH outcross, with only four (of nine) F1 pairs ultimately yielding viable F2 progeny, in widely scattered weeks. The five F1 pairs failing to reproduce included two pairs that laid infertile eggs for 8–9 weeks.

The reproductive delays and reduced fecundities demonstrated in the AH outcross, together with an (apparently natural) accelerated mortality in *Physa hendersoni* adults, combined to yield only 36 F1 progeny (from two sibships) for hybrid testing via allozyme electrophoresis. A fixed difference between the parents of both sibships at the LAP locus allowed unambiguous classification of the 36 individuals as eight offspring from self-fertilization by the *P. acuta* parent, ten offspring from self-fertilization by the *P. hendersoni* parent, and 18 hybrids.

None of our tests comparing *Physa hendersoni* and *P. pomilia* returned evidence of postzygotic reproductive isolation, although we did detect significant life history differences between the control lines (Table 3). *Physa pomilia* control pairs initiated oviposition significantly earlier than either our *P. hendersoni* controls or our HP outcrosses (Fisher's exact p = 0.001 in both cases) with good survivorship over ten weeks of reproduction in all three sets (Fig. 2). Similarly, *P. pomilia* parents demonstrated greater mean weekly fecundity (Kruskal-Wallis $\chi^2 = 8.07$, $p = 0.018$), their 69.4 embryos/week significantly higher than both the 49.2 posted by the *P. hendersoni* controls (Wilcoxon p = 0.043) and the 52.6 posted by the HP outcross pairs (Wilcoxon p = 0.003). No significant differences were detected between the HP outcross and the *P. hendersoni* control in either age at first reproduction or in parental fecundity. Nor were differences detected among all three sets in F1 viability, F1 fertility, or viable F2 hatch.

TABLE 1. Copulations observed in the two mate choice experiments, *P. acuta* x *P. hendersoni* (above) and *P. hendersoni* x *P. pomilia* (below).

		Males			
		Homogametic	Heterogametic	Totals	
Females	*P. acuta* (A)	17	5	22	
	P. hendersoni (H)	13	6	19	
					41
Females	*P. hendersoni* (H)	12	10	22	
	P. pomilia (P)	20	15	35	
					57

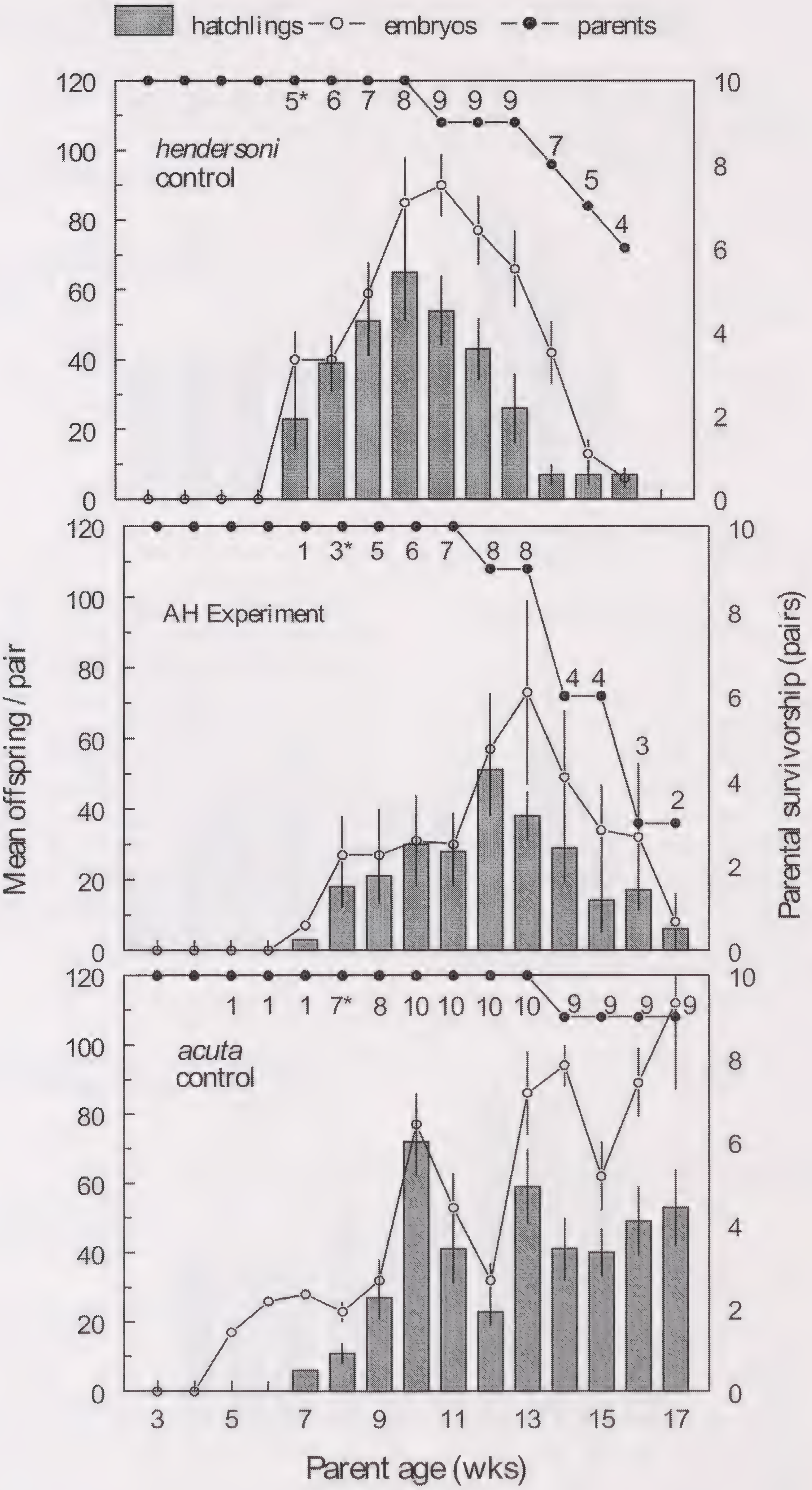

FIG. 1. Production of embryos and viable hatchlings as a function of parental age (weeks post-hatch) in the *P. acuta* x *P. hendersoni* (AH) outcross experiment, pure *P. hendersoni* controls, and pure *P. acuta* controls. The bars are standard errors of the mean. The number of reproducing pairs is given with parental survivorship (right axis). Asterisks* denote week 1 for analysis of variance.

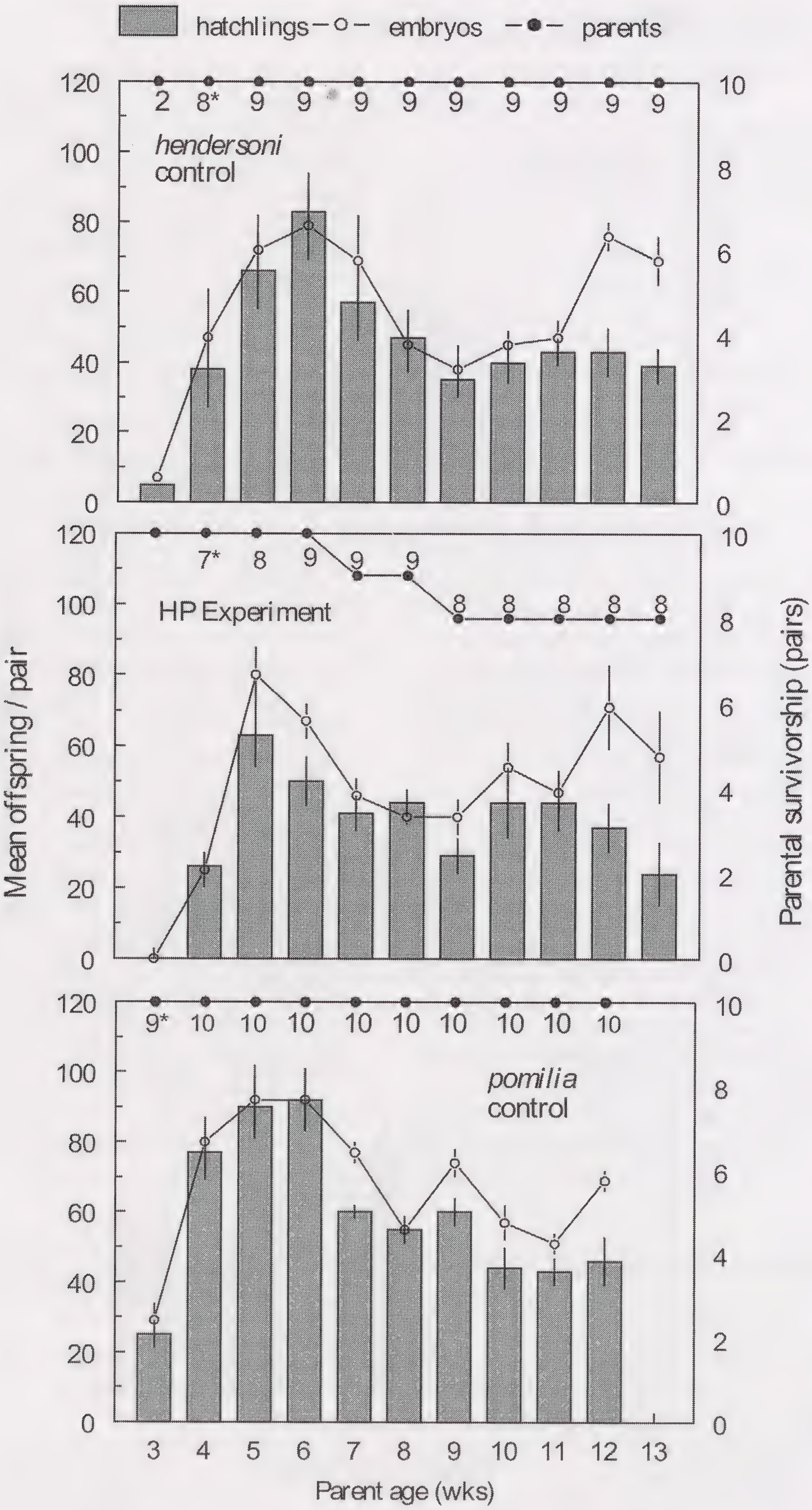

FIG. 2. Production of embryos and viable hatchlings as a function of parental age (weeks post-hatch) in the *P. hendersoni* x *P. pomilia* (HP) outcross experiment, pure *P. hendersoni* controls, and pure *P. pomilia* controls. The bars are standard errors of the mean. The number of reproducing pairs is given with parental survivorship (right axis). Asterisks* denote week 1 for analysis of variance.

TABLE 2. Statistics comparing the fitness of *P. acuta* x *P. hendersoni* outcrosses to pure *P. acuta* and pure *P. hendersoni* controls.

	P. acuta	AH outcross	*P. hendersoni*	p
First oviposition, P generation				0.179
Mode (week)	8	9	7	
Range	5–10	7–16	7–11	
Weekly mean parental fecundity				0.08
Median (embryos)	66.9	24.8	57.4	
Range	17–104	3–96	19–68	
Weekly mean F1 viability				0.756
Median (%)	61.7	67.1	55.8	
Range	45–86	0–91	33–86	
F1 Fertility (%)	100%	44%	78%	
Viable F2 hatch				-
Mode (week)	9	-	5	
Range	9–11	-	3–8	

Electrophoretic analysis of a sample of offspring from three HP outcrosses confirmed the hybrid status of all F1 progeny. Two pairs of parents were (fortuitously) fixed for alternative alleles at the LAP locus, yielding a sample of ten entirely heterozygous progeny in both cases. The progeny of the third pair included six FF homozygotes and six FS heterozygotes, which significantly differs from the 1:2:1 selfed expectation. (The likelihood of sampling no SS homozygotes in 12 trials from such a sibship is 0.032).

DISCUSSION

The data presented here do not support the elevation of *Physa hendersoni* to full specific level (Te, 1978; Burch, 1989). Rather, they confirm the initial proposal of Clench (1925) that the populations described by him as *P. hendersoni* are not specifically distinct from *P. pomilia*. Table 1 demonstrates random mating between snails of our lines H and P. Control populations of *P. pomilia* initially reproduced at an earlier age and showed sig-

TABLE 3. Statistics comparing the fitness of *P. hendersoni* x *P. pomilia* outcrosses to pure *P. hendersoni* and pure *P. pomilia* controls.

	P. hendersoni	HP outcross	*P. pomilia*	p
First oviposition, P generation				0.001
Mode (week)	4	4	3	
Range	3–5	4–6	3–4	
Weekly mean parental fecundity				0.018
Median (embryos)	49.2	52.6	69.4	
Range	0–91	41–70	58–80	
Weekly mean F1 viability				0.128
Median (%)	82.1	78.2	90.2	
Range	0–95	61–97	74–98	
F1 Fertility (%)	100%	100%	100%	
Viable F2 hatch				0.143
Mode (week)	6	6	6	
Range	6–7	5–9	6–7	

nificantly greater fecundity than control *P. hendersoni* under our experimental conditions, but the performance of the HP outcross was not different from that of the *P. hendersoni* control (Table 3). No difference was apparent between the outcross and either of the controls at the F1 or F2 generations.

The distinctions that Clench noted between his new subspecies *Physa pomilia hendersoni* and *P. pomilia* (s.s.) were entirely with regard to minor aspects of shell morphology, the former displaying, "a shorter aperture as compared to the total length of the shell; the aperture more rounded; the spire more produced and acute, and the sutures more deeply impressed." We were not able to confirm these differences, snails from lines H and P being completely indistinguishable to our eyes in culture. It seems likely to us that that the shell differences initially prompting Clench's description of subspecies *hendersoni* may have been ecophenotypic in origin.

Nor do our data support the hypothesis of Te and Burch that *Physa pomilia* should be considered subspecific to *P. heterostropha*, now understood to be a junior synonym of the cosmopolitan *P. acuta*. Rather, our results demonstrate that *P. pomilia* and *P. acuta* are distinct biological species, displaying both prezygotic and postzygotic reproductive isolation. Half of the F1 offspring recovered from no-choice pairings of *P. acuta* and *P. pomilia* were the result of self-fertilization, whereas the rate of self-fertilization is typically found to be negligible in matched controls (Wethington & Dillon, 1993, 1997; Dillon et al., 2005b). Our data further suggest that the other half of the F1 offspring, those that were hybrids, may have been sterile.

At the initiation of our F1 x F1 tests we had no knowledge regarding the hybrid status of the individuals chosen, and hence the nine pairs of F1 snails we selected from the AH lines to assess the production of a viable F2 generation would be expected to include roughly equal proportions of selfed and hybrid animals. Three F1 pairs died relatively early in the experiment, without issue. Two pairs yielded viable F2 offspring early in the experiment, both of which were subsequently shown by allozyme electrophoresis to be composed of one pure-A animal and one pure-H animal. Two pairs yielded viable F2 offspring late in the experiment, both of which proved to be one pure-A animal and one hybrid. And two pairs were terminated late in the experiment by the death of a parent, after weeks of laying infertile eggs. The surviving F1 animal was shown to be a hybrid in both cases. Thus, all viable F2 offspring ultimately recovered from the AH experiment are attributable to reproduction by purebred F1 animals, and our results are consistent with a hypothesis of *P. acuta* x *P. pomilia* hybrid sterility.

The significant tendency of animals from our H and A lines to select mates from their own populations (Table 1, upper) is the second demonstration of prezygotic reproductive isolation in basommatophoran pulmonates of which we are aware. Similar phenomena have previously been documented in stylommatophoran pulmonates (Baur & Baur, 1992; Fearnley, 1996) as well as in the intertidal caenogastropod snail *Littorina* (Pickles & Grahame, 1999; Rolan-Alvarez et al., 1999; Cruz et al., 2004). But the only prior report of prezygotic reproductive isolation in freshwater pulmonates, to our knowledge, is that of Rupp & Woolhouse (1999) working with *Biomphalaria glabrata* and *B. pfeifferi*.

Clench gave the distribution of "typical" *Physa pomilia* as "extending as far north as Kentucky and west to Mississippi." He suggested that his new subspecies *P. pomilia hendersoni* "seems to be confined to the coastal area of the Southeast." Our personal observations, although more limited, tend to agree. *Physa pomilia* seems moderately common in Atlantic drainages from Virginia to Georgia, especially in coastal rivers of low current. Although it is clear that the species extends as far south as Alabama and as far north as Connecticut (Wethington & Lydeard, in press), we do not have good field records beyond the southern Atlantic drainages. The situation is complicated by confusion with *P. acuta*, which is common and widespread throughout North America, and which can be difficult to distinguish from *P. pomilia* without dissection. A third species of physid also inhabits swampy coastal environments of the American Southeast and may also be confused with *P. pomilia*. Work to characterize this third species is currently ongoing.

Our data on reproductive isolation agree well with the results of recent studies reporting mitochondrial sequence divergence in large samples of physids surveyed from throughout North America (Wethington & Guralnick, 2004; Wethington & Lydeard, in press). Molecular phylogenetic analysis has confirmed that *Physa hendersoni*, *P. pomilia*, and other type-bc physids are closely related, and that type-bc physids are genetically distinct from both the type-b and type-c groups. Additional studies to characterize reproductive relationships in earlier branches of the phylogeny could yield valuable insight into the mechanics of speciation, not just in the Physidae but throughout the invertebrate fauna of fresh waters.

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SPECIES DELIMITATION IN THE GENUS *BYTHINELLA* (MOLLUSCA: CAENOGASTROPODA: RISSOOIDEA): A FIRST ATTEMPT COMBINING MOLECULAR AND MORPHOMETRICAL DATA

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ABSTRACT

Within the springsnail genus *Bythinella*, few discontinuous morphological characters allow to unambiguously delineate species-level taxa. Opinions on the alpha-taxonomy of the group are divergent, with some authors recognizing every morphologically distinct local form as a species, while others interpreting such geographical forms as intraspecific variation. Because the value of morphological characters was rarely contrasted with molecular data, such opinions remained untested. In this work, variation between populations was studied through genetics isozymes, phylogeny (DNA), and morphometrics. Eleven populations representing five putative species were sampled from the French Pyrenees, a region where a high number of nominal species are classically recognized. Based on genetic and phylogenetic analyses, the material clusters into three groups, of which one consists of several nominal species. Environmental factors, sexual dimorphism, and genetic factors contribute to the significant morphological variation observed within the genetic groups. Thus, the number of species of *Bythinella* recognized in the Pyrenees appears probably overestimated, and the characters traditionally used for species delimitation should be re-evaluated.

Key words: hydrobioid springsnails, ITS-1 nuclear gene, isozyme, phylogeny, morphometry, multivariate analyses.

INTRODUCTION

Among gastropods, hydrobioid springsnails are one of those taxa in which the lack of obvious discriminating morphological characters makes species delimitation difficult, resulting historically in the establishment of numerous nominal species and currently in a variety of specialist opinion on the taxonomic validity of these taxa (Kabat & Hershler, 1993; Herschler & Ponder, 1998; Wilke et al., 2001; Szarowska & Wilke, 2004). A consequence is that different specialists have different evaluations of the magnitude of biodiversity. The difficulty of resolving such differing opinions is also hampered by the lack of a solid theoretical and methodological framework, *de facto* rendering many taxonomic opinions untestable hypotheses. However, and although delimitating species boundaries is a central aim of alpha-taxonomy, there is no consensus concerning the meaning of the term "species" (as instances of the extensive literature on this question, see, e.g. Howard & Berlocher, 1998; Wilson, 1999; Winston, 1999; Wheeler & Meier, 2000; Hey, 2001a, b; Mallet, 2001; Noor, 2002).

The objective of this paper is to test the validity of the traditional delimitation of some species belonging to the genus *Bythinella* Moquin-Tandon, 1856, based upon a species concept that permits testable hypotheses. For this purpose, we use the Hennigian inter-nodal species concept recently developed and formalized by Samadi & Barberousse (2006). This species definition is close to the Evolutionary Species Concept (Simpson, 1961, modified by Wiley, 1981; Wiley & Mayden,

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2000), the Internodal Species Concept (Kornet, 1993) and the General Lineage Species Concept (De Queiroz, 1998, 1999). For De Queiroz (1998), most “species concepts” are ill-named, because they should rather be regarded as recognition criteria that, associated with a formal definition of the “species” that is independent of the mechanism by which species are recognized, permit delimitation of species in practice. As suggested by these authors, the various techniques used in systematics can be set in a coherent framework starting from species descriptions based on morphology and/or behavioural and ecological attributes. These descriptions are the *primary hypotheses* for species delimitation that correspond to alpha-taxonomy. Second, these hypotheses can be tested against criteria derived from the various so-called “species concepts”, such as the Phylogenetic Species Concept (PSC) and/or the Biological Species Concept (BSC), corresponding respectively to the approaches of phylogenetic systematics (*sensu* Hennig, 1966) and evolutionary systematics (*sensu* Mayr, 1942). Finally, the results of these tests permit the selection of character sets corresponding to more robust primary descriptions. Here we attempt to delimit some *Bythinella* species, in a restricted geographical area, in coherence with the theory of evolution and to justify why, as argued for example by Sites & Marshall (2003, 2004), we need an integrated approach in taxonomy.

Bythinella species live in emerging groundwater springs and in the uppermost courses of small streams, where they can form large populations (1,000–10,000s individuals). Some taxa live exclusively in groundwater (Giusti & Pezzoli, 1980; Falniowski, 1987; Bernasconi, 2000; Bertrand, 2004; Bichain et al., 2004). The small (usually around 2.5 mm, maximum 4 mm), nondescript shell is characterized by a blunt apex, with an overall ovoid to conical-elongate shape, most often smooth, but occasionally with spiral keel(s) or one or more axial varices on the last adult whorl. Species of *Bythinella* are gonochoristic. Males are characterized anatomically by a penis-flagellum complex; females have a bursa copulatrix and a seminal receptacle. France is an area of high specific richness for the genus. Of the species currently recognized in Europe, 62% occur in France, of which 90% are endemic to France (Fauna Europaea, 2004).

In *Bythinella*, as in the majority of hydrobioid (*sensu* Davis, 1979) genera, species are primarily delimited based on non-discrete characters. A consequence of this continuous variation of character states is a range of taxonomic opinions among the various taxonomic authorities. For example, in Italy, based on shell morphology and reproductive anatomy, Alzona (1971) recognized eight species of *Bythinella*, whereas Giusti & Pezzoli (1980) regarded all Italian populations as belonging to two species. Based on shell characters, Radoman (1976) recognized about 12 species in the Balkans and Asia Minor. Based on shell morphology, soft part anatomy, and geographical distributions, Falkner et al. (2002) recognized 42 valid species in France, with several applications of species names differing from those of Bernasconi (2000). Thirteen of these species were restricted to the Pyrenees and their foothills. However, in Poland analyses of enzyme polymorphism and morphometric studies performed on shell and anatomical characters (Falniowski et al., 1998, 1999; Mazan, 2000; Mazan & Szarowska, 2000a, b) questioned the ability of the shell and anatomical characters used in alpha-taxonomy to delineate species of *Bythinella*. Consequently, because of these various opinions, which recognize these taxonomic entities alternatively as “good species” or not, we have no clear idea of the true diversity of this group.

Because of their narrow ranges and specialized habitat, hydrobioid springsnails in general, and *Bythinella* species in particular, are vulnerable to even small-scale habitat transformation, such as trampling by cattle or artificial diversion of springs. Several hydrobiid species are considered extinct, and many others are categorized as threatened (IUCN 2005, http://www.redlist.org). Of the species occurring in France, nine are nationally protected (statutory order October 7, 1992). Legal protection was bestowed upon them based on the state of taxonomic knowledge in the 1980s, and it is now fitting to evaluate whether the names involved in the legal texts apply to real units of biodiversity. Of the putative species analyzed in the present study, two are nationally protected.

Species delimitation in a number of nominal *Bythinella* species from the Pyrenees was explored according to criteria derived from the PSC and the BSC, and our purpose was to test if sets of morphological characters (traditional or newly developed) correspond to these criteria. For this purpose, we used populations representing nominal species sampled from their type localities or from the nearest possible area if the precise type locality could not be found or no longer existed. We performed multivariate analyses on shell parameters to evaluate whether the pattern of shell variation within and among populations permits non-ambiguously attributing each of the sampled population to a putative species, and we tested species delimitation resulting from traditional morphological approaches against BSC and PSC criteria. The BSC criterion amounts to delimitation of species as reproductively isolated groups that became genetically isolated from other such groups. Isolation was evaluated indirectly through analysis of gene flow among populations using electrophoretic variability of isozymes. In the most common view of the PSC, species are monophyletic groups. Monophyly of groups was identified using the pattern of variability of the ITS-1 nuclear gene. These three approaches were compared in order to evaluate the potential use of the morphological characters in the delimitation of *Bythinella* species.

MATERIAL AND METHODS

Taxa and Populations Studied

The taxa selected for the present study essentially originate from the Département Ariège, in the central/northeastern Pyrenees. The area has extensive karsts, with numerous springs where *Bythinella* species live in dense populations. Based on shell and reproductive anatomy, Bernasconi (2000) had proposed an alpha-taxonomy of *Bythinella* from this area, and we followed his treatment in how we applied specific names to each of the populations sampled.

Eleven populations were sampled that, based on Bernasconi (2000) and Falkner et al. (2002), correspond to five species (Table 1, Fig. 1). Ten of these populations, representing four putative species, are from the Pyrenean foothills, but *B. reyniesii* (Dupuy, 1851) is a higher altitude species. *Bythinella simoniana* (Moquin-Tandon, 1856), a species relatively well characterized by one or more axial varices on the body whorl, was sampled from three populations (Eng, Cat and Sou). Four populations (Tdl1, Tdl2, Suz and Roq) are attributable to *B. utriculus* (Paladilhe, 1874).

At Audinac, we sampled *Bythinella* from a thermal spring (ca. 18–20°C; Aud1), from a cold spring (ca. 13°C; Aud2) just 30 meters away, and from the confluence (Aud3) of the two springs. The thermal spring is the type locality of *B. rubiginosa* (Boubée, 1833), a nominal species considered to be restricted to this locality; the application of the species name is thus unambiguous. The population sampled from Aud2 had been identified by Bernasconi (2000) as *B. eurystoma* (Paladilhe, 1870). However, specimens do not match the original description of this nominal species well (type locality is St Jean-de-Fos, Département Hérault); we consequently do not follow Bernasconi in applying this name to that population, which we will from hereon designate as *B.* cf. *eurystoma*. The third population (Aud3) was not included in Bernasconi's dataset, and we could not easily place it in one of the nominal species.

As a geographical out-group within *Bythinella*, we used *B. viridis* (Poiret, 1801), the type species of the genus, which we sampled from the type locality (Che) in the east of the Paris basin. It has an ovoid shell that clearly sets it apart from all the Pyrenean *Bythinella* species included in our study.

Specimens were collected by washing small pebbles, aquatic vegetation and dead leaves over two sieves (2 mm and 450 µm mesh). For anatomical and biometrical studies, specimens were fixed in 70% ethanol; for molecular studies, specimens were frozen alive at -80°C. Given the very small size of the specimens, each of the different analyses (morphometric, isozyme and DNA sequence analyses) could not be carried out on the same individuals.

Isozyme Electrophoresis

Protein extraction was carried out on whole animals (including the shell), and followed the protocol of Boisselier-Dubayle & Gofas (1999). Electrophoresis was done using vertical acrylamide gels on discontinuous systems. The running buffer was tris-glycine (4.95 mM, pH 8.3). Of the eight enzyme systems assayed, three gave scorable banding patterns and are used in this study (phosphoglucomutase: *Pgm*, EC 5.4.2.2; glucose-6-phosphate isomerase: *Gpi*, EC 5.3.1.9; aspartate aminotransferase: *Aat*, EC 2.6.1.1). Because of the small size of the specimens, it was technically not feasible to study all three enzyme systems on the same individual. We therefore extracted between 30 and 40 individuals per population, giving approximately 20 for each loci. In total, 439 individuals were used for the analyses (Table 1).

TABLE 1. *Bythinella* populations characteristics and number of individuals used for each analysis. All localities were cited by Bernasconi (2000). Tdl1 and Tdl2 are populations in two adjacent springs separated by 10 meters. Both fall within the generalized type locality of *Bythinella utriculus*. N_{ea} = number of individuals used for enzymatic activities, N_{sh} = number of shells measured.

	Population information						ITS-1 sequences		
Nominal species	Code	Type Locality	Localities	Coordinates	Altitude (in meters)	N_{ea}	DNA isolate	GenBank number	N_{sh} and MNHN number
B. viridis	Che	yes	Chery-Chartreuve (02) Moulin de Veau	3°37'51"E 49°15'45"N	130	25	Che1 Che3	DQ318901 DQ318902	27 Moll5959
B. rubiginosa	Aud1	yes	Audinac-les-Bains (09) thermal spring	1°10'56"E 43°00'22"N	450	35	Aud11 Aud12	DQ318905 DQ318906	30 Moll5960
B. reyniesii	Por	no	Boussenac (09) Col de Port	1°27'7"E 42°53'58"N	1250	38	Por1 Por2	DQ318899 DQ318900	30 Moll5961
B. simoniana	Cat	no	Alas (09) Sainte-Catherine	1°01'23"E 42°57'19"N	510	36	Cat3	DQ318903	30 Moll5962
	Eng	no	Engomer (09) Arguilla	1°04'17"E 42°56'53"N	460	38	No sequenced		30 Moll5963
	Sou	no	Clermont (09) La Souleille	1°16'48"E 43°01'29"N	300	40	Sou2	DQ318904	30 Moll5964
B. cf. *eurystoma*	Aud2	no	Audinac-les-Bains (09) no thermal spring	1°10'56"E 43°00'22"N	450	38	Aud23 Aud26	DQ318907 DQ318908	30 Moll5965
Bythinella sp.	Aud3	no	Audinac-les-Bains (09) confluence of AUD1 and AUD2	1°10'56"E 43°00'22"N	450	34	Aud32 Aud31	DQ318909 DQ318910	30 Moll5966
B. utriculus	Suz	no	La Bastide-de-Sérou (09) Col de Suzan	1°25'10"E 43°01'16"N	410	34	Suz3 Suz5	DQ318914 DQ318915	30 Moll5967
	Tdl1	yes	La Bastide-de-Sérou (09) Tour de Loly	1°27'16"E 43°00'56"N	460	37	Tdl15 Tdl12	DQ318912 DQ318913	30 Moll5968
	Tdl2	yes	La Bastide-de-Sérou (09) Tour de Loly	1°27'16"E 43°00'56"N	460	42	No sequenced		29 Moll5969
	Roq	no	Roquefort les Cascades (09) Cascades des Turasses	1°45'44"E 42°57'22"N	460	33	Roq2	DQ318911	59 Moll5970

Two polymorphic loci were interpreted for *Pgm*, only one for *Aat* and *Gpi*. Multiple loci encoding the same enzyme were numbered in order of decreasing mobility. The alleles were numbered with the same system.

For the four putative loci, data were scored in a matrix of individual genotypes. For each population, allele frequencies at each locus, percentage of polymorphic loci at 0.95 (*P*), mean number of alleles per locus (*A*), mean observed and expected heterozygosities (*Ho* and *He*), pairwise theta (Weir & Cockerham, 1984) among populations (θ) were estimated using Genetix version 4.02 (Belkhir et al., 2001). Departures from Hardy Weinberg equilibrium (HWE) were tested in two steps: first a global test was performed using the exact HW test of Haldane (1954), Weir (1990), and Guo & Thompson (1992); second, heterozygote deficit or excess was tested with U tests. Genotypic linkage disequilibria were measured within each population and for each pair of locus, and exact tests of genotypic differentiation were performed per locus and for each population pair. All these tests were performed with Genepop version 3.4 (Raymond & Rousset, 1995). A sequential Bonferroni correction (Holm, 1979; Rice, 1989) was used when several statistical tests were performed simultaneously (for HWE, heterozygote deficit or excess, linkage disequilibrium).

A dendrogram of the 12 populations was constructed to analyse the distribution of genetic variation. The chord distance (Cavalli-Sforza & Edwards, 1967) was computed between all pairs of populations and the popu-

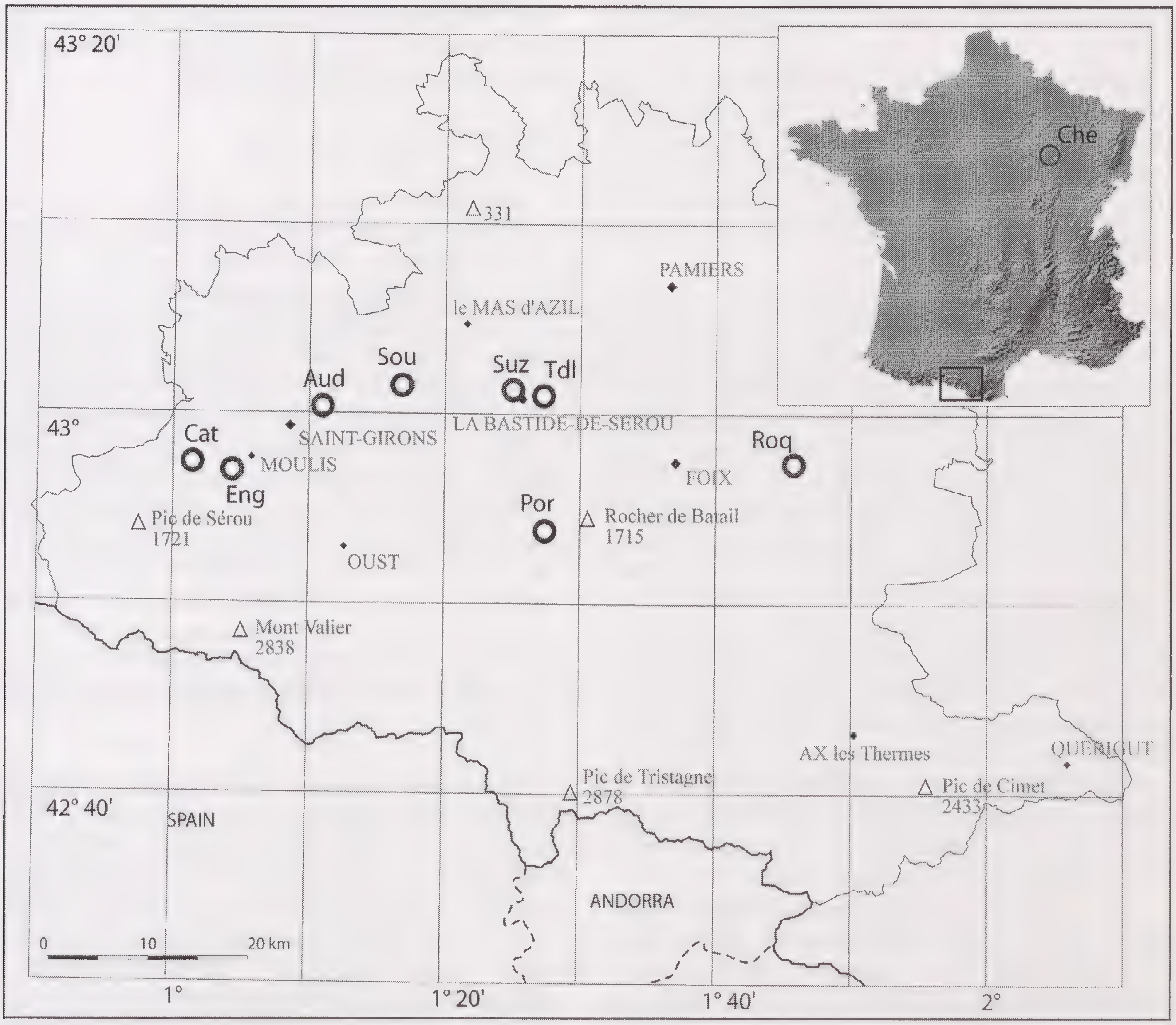

FIG. 1. Geographical location of *Bythinella* populations sampled. For details of the contiguous stations from Audinac (Aud) and La-Bastide-de-Sérou (TdL), see Table 1.

lations were clustered using the neighbour-joining (NJ) method. The chord distance assumes that allele frequency changes result from genetic drift alone; it does not assume that population sizes have remained constant and equal in size (Felsenstein, 1993). This analysis was conducted using Populations version 1.2.28 (Langella, 2002).

DNA Techniques and Phylogenetic Analyses

The ribosomal internal transcribed spacers (ITS-1 and ITS-2) are intergenic DNA sequences that, because of a relatively fast rate of evolution, are useful in resolving phylogenetic relationships of closely related taxa (see, for example, Schilthuizen et al., 2004, for molluscs, or for other zoological groups see (Hillis et al., 1996; Coleman, 2003; Chen et al., 2004). We here use the variability of ITS-1 that lies between ribosomal nuclear genes 18S and 5.8S.

DNA was extracted from whole individuals including shells, using the QIAGEN DNeasy®96 kit. The entire ITS-1 region was amplified using two universal primers ITS-2 (5'-GCT GCC TTC TTC ATC GAT GC-3') and ITS-5i (5'-AGG TGA CCT GCG GAA GGA TCA TT-3') (Hillis & Dixon, 1991).

PCR reactions were performed in a final volume of 50 µl, using approximately 5 ng of template DNA, 2.5 mM $MgCl_2$, 0.6 µM of each primer, 0.26 mM of each nucleotide, 5% DMSO and 1.5 unit of *Taq* polymerase (Qbiogene). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 40 cycles at 94°C for 30 s, 50°C for 30 s and 30 s at 72°C, and a final extension at 72°C for 5 min. PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced on a Ceq2000™ automated sequencer (Beckman), in both directions to confirm accuracy of each sequence. We obtained 17 sequences corresponding to one or two individuals analysed for each population (Table 1), aligned with the ClustalW algorithm (Thompson et al., 1994) implemented in the BioEdit 5.0.0 sequence alignment editor software package (Hall, 1999).

The DNA fragment sequenced included several highly variable regions with numerous insertions/deletions. These variable regions were removed from the data set and manually aligned following the procedure of Barriel (1994): number of indels, number of changes and number of modified sites are minimized in this order of priorities, a transition being preferred to a transversion in the ambiguous areas. Each plausible alignment for each of the highly variable regions was tested with PAUP* 4.0b (Swofford, 2000). The criterion used to choose an alignment among the different alignments tested was to obtain the most parsimonious tree.

After removing the first 5' ambiguous variable region which was located between the RNA18S gene and the first ITS-1 conserved region, the final data matrix was composed of 17 sequences and 360 bp. A maximum parsimony analysis was performed using PAUP*4.0b. Gaps were included as missing data. The analysis was performed using a heuristic search with 100 random-addition replications, branch swapping by the Tree Bisection and Reconnection (TBR) algorithm. To test the robustness of the results, the Bremer index (Bremer, 1994) was calculated and 100 bootstrap replicates were carried out, using the same heuristic search settings.

Morphometrical Analysis

Shell Measurements – Between 30 and 59 adult shells were used per population. Shells were washed in a 5% Chlorox solution for 30 min, then rinsed in distilled water, transferred to absolute ethanol and dried. The shells were placed on an adhesive support in a standardized position (for more details, see the legend of Fig. 2) and then digitized with a graduated scale using a stereomicroscope associated with a digital camera. An orthonormal frame (Ox, Oy) and the aperture center were overlaid on the digital picture, and 12 landmarks were then positioned using the TpsDig 1.23 software package (Rohlf, 2001). The coordinates of these landmarks within the orthonormal frame (Ox, Oy) were used to take 15 measurements characterizing individual shells (Fig. 2).

Sex Determination – Sexing the animal required dissolving the shell for dissection. After digitalization, specimens were removed from the adhesive support, placed for four min in a solution of 5% hydrochloric acid and 70% ethanol for shell dissolution, and then rinsed in distilled water. Dissections were done under a dissecting stereomicroscope. Sex was determined by the presence of the flagellum-penis complex for males and by the presence of the albumen and capsule glands for females.

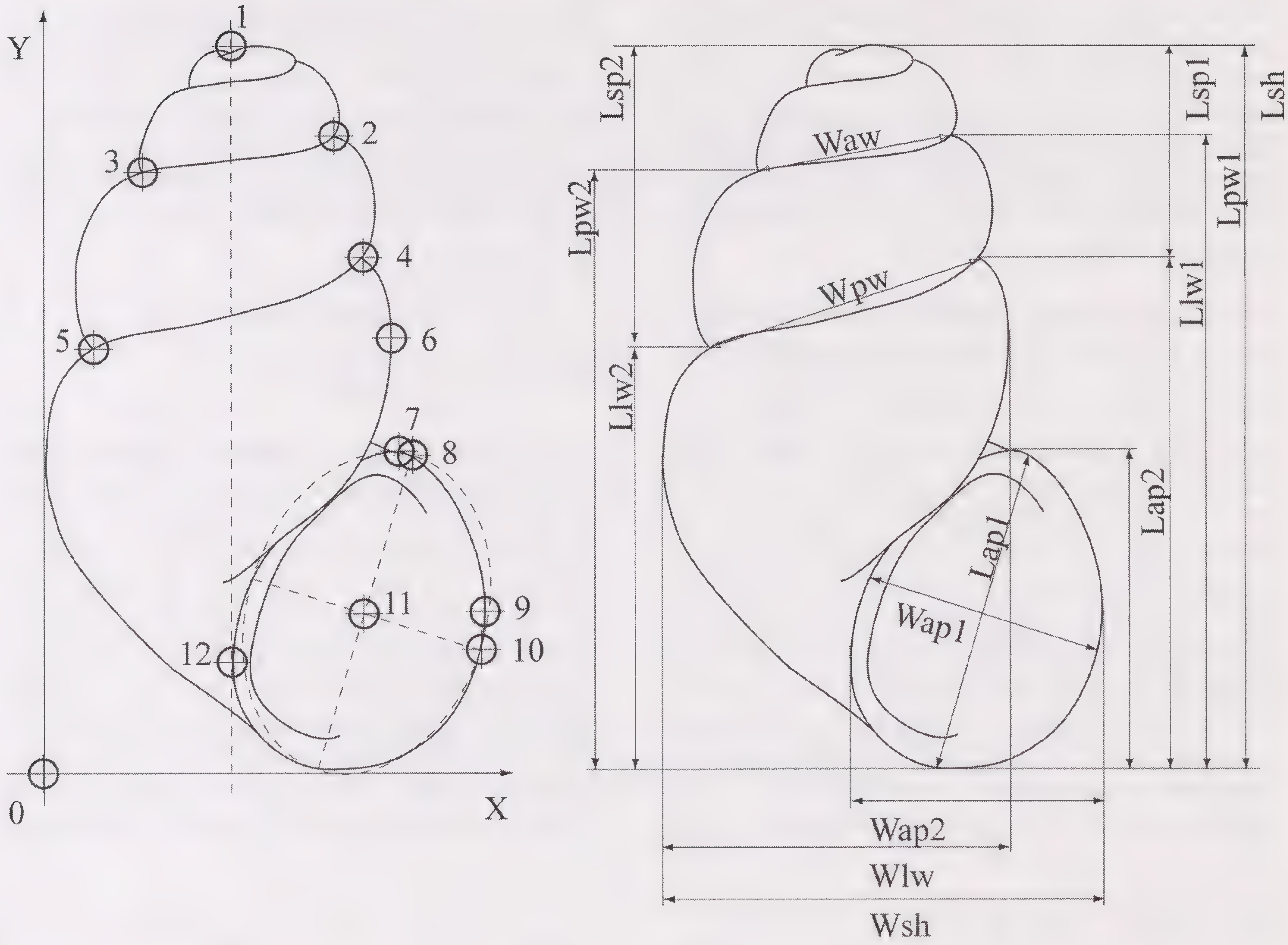

FIG. 2. Morphological parameters taken from shell of *Bythinella* in standard position: the collumelar axis and the aperture plane are parallel to the horizontal plane. Left – Landmarks locations. Orthonormal frame (Ox, Oy): the Oy axis is parallel to the collumelar axis and tangential to the left edge of the shell. The *Ox* axis is perpendicular to *Oy* axis and tangential to the lower edge of the aperture. The aperture center is the center of the *E* ellipse which is adjusted with the aperture edge. Right – Measurements taken. Shell height (L_{sh}), aperture length ($L_{ap1} = 2\sqrt{[x_8-x_{11})^2+(y_8-y_{11})^2]}$), aperture height ($L_{ap2}$), aperture diameter ($W_{ap1} = 2\sqrt{[x_{10}-x_{11})^2+(y_{10}-y_{11})^2]}$, aperture width ($W_{ap2} = x_9-x_{12}$), shell width ($W_{sh}$), width of last whorl ($W_{lw} = x_6$), width of penultimate whorl ($W_{pw} = \sqrt{[x_4-x_5)^2+(y_4-y_5)^2]}$), width of antepenultimate whorl ($W_{aw} = \sqrt{[x_2-x_3)^2+(y_2-y_3)^2]}$). Height measurements of the spire and of each whorl were taken on the left and on the right sides of the shell: left and right spire heights ($L_{sp1} = y_1-y_2$, $L_{sp2} = y_1-y_5$), left and right heights of penultimate whorl (L_{pw1}, L_{pw2}), left and right heights of last whorl (L_{lw1}, L_{lw2}).

Analysis of Sexual Dimorphism – Sexual dimorphism in *Bythinella* had been reported by Falniowski (1987) but never quantified. We assessed first how shell dimorphism might interfere with species delimitation within populations. For this, we analyzed shell parameters in 59 individuals from Roq. The input data in millimeters were transformed into Log-shape Ratio (LSR) data in order to limit an eventual size effect (Mosimann, 1970). We first performed a Principal Component Analysis (PCA) to explore how the range of shell parameters was distributed between males and females. Then a Discriminant Function Analysis (DFA) was used to test statistically the differences between males and females and then to identify which shell parameters reflect sexual dimorphism. Wilks' Lambda (WL) used in an ANOVA (F) test of mean differences was used to test if the discriminant model as a whole was significant. Then, if the global F test was significant, each variable was tested using Wilks' lambda to determine which variable differed significantly in mean between discriminated groups.

Second, we evaluated the effect of sexual dimorphism among populations, taxonomic groups and genetic groups. For this purpose, an analysis of variance (ANOVA) with two fixed factors (population and sex) was carried out on the parameters revealed by the DFA as significantly contributing to shell dimorphism in the population analysed. This analysis permitted comparison of the relative effects of sex and population location on shell shape, and identification of the interaction between these two factors. Tukey's HSD *post hoc* test and its associated probability were used to examine the statistical significance of the differences between all pairs of means.

Analysis of Shell Variation Between Populations and Taxa – DFAs and associated Wilks' Lambda were performed on all 15 measurements after LSR transformation, and two discriminant factors were tested: (i) the *Taxon* discriminating factor: populations referred *a priori* to a nominal species based on the collection site were tested *a posteriori* to check whether they could be discriminated based on shell parameters (population Aud3 was excluded from the analysis because it was not assigned to a nominal taxon); (ii) the *Genetic* discriminating factor: clusters of populations resulting from the genetic and phylogenetic analyses were tested *a posteriori* to check whether specimens grouping together could also be discriminated based on their shell. We then compared the reclassification scores of the discriminating linear functions of the two grouping methods (i.e., taxon or genetic groups).

All analyses were performed with the software package Statistica 6.0 (Statsoft, 2001) and the level of significance used was 5%.

RESULTS

Isozymes Polymorphism

The three enzyme systems involving four putative loci revealed from three to five alleles per locus (Table 2), and the mean number of alleles per population ranged from 1.0 to 2.25 (Table 3). The *Aat-1* locus was the least polymorphic, with a total of three alleles overall and with each population being monomorphic. The one population (taxon) used as the out-group (*Bythinella viridis*) was monomorphic for all four loci, as was the Sou population. For the other populations, the mean observed heterozygosity (*Ho*) ranged from 0.015 to 0.241. Genotype

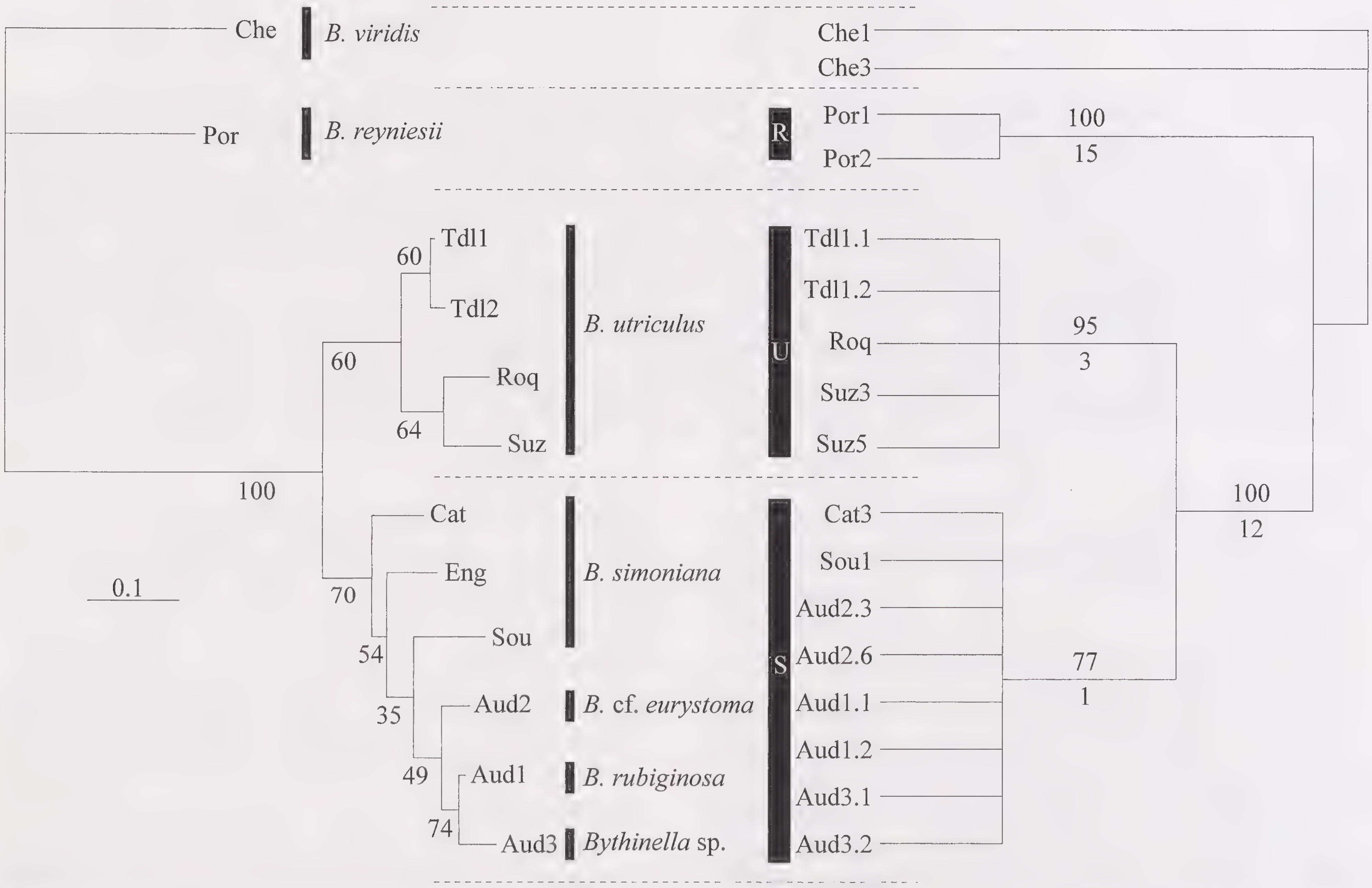

FIG. 3. NJ tree constructed from isozyme data on the D chord distance (Cavalli-Sforza & Edwards, 1967) between populations of *Bythinella* (left) with the Bootstrap values and parsimony tree (strict consensus) computed from ITS-1 sequences (right) with a heuristic search (Bootstrap values above, Bremer index below).

TABLE 2. Allele frequencies observed at 4 loci on 12 populations of *Bythinella*. N = number of individuals analysed for each locus.

		Aud1	Aud2	Aud3	Cat	Eng	Sou	Tdl1	Tdl2	Roq	Suz	Por	Che
Pgm-1	(N)	(24)	(24)	(23)	(24)	(22)	(28)	(25)	(25)	(24)	(24)	(22)	(7)
	1	-	-	-	-	0.023	-	-	-	-	-	1.000	-
	2	0.417	0.458	0.674	0.250	0.068	-	0.140	0.100	-	0.063	-	-
	3	0.563	0.458	0.326	0.688	0.886	1.000	0.340	0.300	0.854	0.208	-	-
	4	0.021	0.083	-	0.063	0.023	-	0.520	0.600	0.146	0.729	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	1.000
Pgm-2	(N)	(24)	(24)	(18)	(24)	(18)	(28)	(21)	(20)	(19)	(19)	(17)	(9)
	1	1.000	1.000	1.000	0.646	0.889	1.000	0.262	0.225	-	-	0.029	-
	2	-	-	-	0.354	0.111	-	0.738	0.775	1.000	1.000	-	-
	3	-	-	-	-	-	-	-	-	-	-	0.971	-
	4	-	-	-	-	-	-	-	-	-	-	-	1.000
Gpi-1	(N)	(24)	(25)	(22)	(27)	(22)	(30)	(27)	(30)	(26)	(26)	(24)	(19)
	1	-	-	-	-	-	-	-	-	-	-	1.000	1.000
	2	0.958	1.000	0.955	0.981	0.932	1.000	1.000	1.000	1.000	1.000	-	-
	3	0.042	-	0.045	0.019	0.068	-	-	-	-	-	-	-
Aat-1	(N)	(9)	(8)	(12)	(12)	(17)	(16)	(10)	(14)	(1)	(1)	(14)	(10)
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	-
	2	-	-	-	-	-	-	-	-	-	-	1.000	1.000

TABLE 3. Genetic variability computed on the four analysed loci on *Bythinella* populations. *Ntot* = total number of individuals used for isozyme activities; *n* = mean number of individuals analysed per locus; *P* = percentage of polymorphic loci; *A* = mean number of allele per locus; *Ho* = observed heterozygosity; *He* = expected heterozygosity; *HWE* = Hardy-Weinberg equilibrium tests (Fischer's method for test of significance, *significant at 0.05 level).

Population	*Ntot*	*n*	*P*	*A*	*Ho*	*He*	*HWE*
Aud1	35	20.3	0.5	1.75	0.104	0.150	0.22
Aud2	38	20.3	0.25	1.5	0.146	0.146	1
Aud3	34	18.8	0.5	1.5	0.164	0.135	0.712
Cat	36	21.8	0.75	2.0	0.134	0.244	0.003*
Eng	38	19.8	0.75	2.25	0.124	0.137	0.146
Sou	40	25.5	-	1.0	-	-	-
Tdl1	37	20.8	0.5	1.75	0.241	0.251	0.179
Tdl2	42	22.3	0.5	1.75	0.162	0.227	0.158
Roq	33	17.5	0.25	1.25	0.052	0.064	0.398
Por	38	19.3	0.25	1.25	0.015	0.015	1
Suz	34	17.5	0.25	1.5	0.073	0.107	0.089
Che	25	11.3	-	1.0	-	-	-

TABLE 4. F-statistics indices (Weir & Cockerham, 1984) calculated for the *Bythinella* populations analysed (excluding out-group and Por populations, see text for explanations).

Locus	F_{IS}	F_{ST}	F_{IT}
Pgm-1	0.209	0.332	0.472
Pgm-2	0.179	0.718	0.769
Gpi-1	-0.033	0.020	-0.012
all	0.189	0.481	0.579

frequencies generally matched Hardy Weinberg expectations after a sequential Bonferroni correction, except for the Cat population, which had a heterozygosity deficit. No genotypic linkage disequilibrium between pairs of loci was found.

These four loci were together diagnostic for two of the six presumptive taxa: (i) individuals from the type-locality of *B. viridis* had diagnostic alleles for the two loci *Pgm-1* and *Pgm-2*; and (ii) individuals from Por, attributed to *B. reyniesii*, had a diagnostic allele for *Pgm-2*. Furthermore, individuals attributed to *B. viridis* and *B. reyniesii* together differed from all other *Bythinella* specimens by the same diagnostic alleles at the two loci *Gpi-1* and *Aat-1*. All other populations sampled from the Pyrenean foothills shared alleles with different frequencies at all the loci scored. Thus, gene flow between any of these three groups and any other population from our sample can be excluded, whereas gene flow among populations from the Pyrenean foothills cannot be excluded.

The *NJ* tree constructed on the chord distance illustrates the genetic relationships between populations (Fig. 3, left). Two populations, *B. viridis* and the Por population (attributed to *B. reyniesii*) were highly divergent from all others. For other populations, the topology of the tree revealed two main genetic groups. First, the four populations Tdl1, Tdl2, Suz, and Roq (attributed to *B. utriculus*) clustered together, with values of *D* ranging from 0.001 (Tdl1 *vs.* Tdl2) to 0.1 (Tdl1 *vs.* Roq). Second, the populations Aud1 (*B. rubiginosa*), Aud2 (*B.* cf. *eurystoma*), Aud3, Sou, Eng, and Cat (the last three attributed to *B. simoniana*) clustered together, with *D* ranging from 0.011 (Aud1 *vs.* Aud2) to 0.139 (Aud3 *vs.* Sou).

Because the pattern of shared alleles indicated that gene flow could not be excluded among the Pyrenean foothill populations, we analysed the genetic structure among them using F statistics (Table 4). The out-group and the Por population of *B. reyniesii* were not included in this analysis. The genetic variance among populations (F_{ST} = 0.481) represented the principal component of the total genetic variance of the populations analysed (F_{IT} = 0.579); the genetic variation within populations was relatively low (F_{IT} = 0.189). Thus, these *Bythinella* populations are highly differentiated genetically. The θ values estimated between pairs of populations were significantly different from zero, except between populations collected in the same locality (Aud1, Aud2, Aud3; Tdl1, Tdl2) or nearby (Tdl2 with Suz). The genotypic differentiations calculated for each pair of populations and for all loci reveal significant *P* values, except between populations located in the same locality (Aud1 to 3 and Tdl1 and 2).

Phylogenetic Analysis

Of the 360 bp analysed, 76 positions were variable and 48 were phylogenetically informative. Figure 3 (right) displays the strict consensus tree based on 2,700 equi-parsimonious trees resulting from the heuristic search. The tree length was 96 steps with CI = 0.96 and RI = 0.96.

The topology revealed three distinct clades. The first clade (clade R) included the individuals from Por referred to *B. reyniesii*. This clade was supported by strong Bootstrap values (= 100) and Bremer index (= 15). The second clade (clade U) included the individuals from populations referred to *B. utriculus* (Tdl1, Suz and Roq). The third clade (clade S) included the individuals referred to *B. rubiginosa* (Aud1), *B.* cf. *eurystoma* (Aud2), *B. simoniana* (Cat and Sou), and the Aud3 population. Clade U and S constituted a monophyletic group, which was also supported by strong Bootstrap values (= 100) and Bremer index (= 12).

The phylogenetic analysis corroborated the enzyme polymorphism studies. Indeed, the three monophyletic groups revealed corresponded to the *B. reyniesi* population and the two main groups revealed by the isozyme analyses. Therefore, in the following morphological analysis, we consider these groups as a hypothesis alternative to the classical taxonomy, which can be formulated as follows: four population groups of our dataset constitute four distinct species: Group V = Che (used as out-group); Group R = Por; Group S = Aud1, Aud2, Aud3, Cat, Sou and Eng; Group U = Tdl1, Tdl2; Suz and Roq.

Morphometrical Analysis

Sexual Dimorphism – Overall 358 specimens were successfully sexed, 55% males and 45% females. Within a single population, 59 specimens from Roq included 36 (61%) males and 23 (39%) females. PCA was carried out on the 15 shell parameters LSR transformed. The first (PC1) and second (PC2) principal components respectively account for 63.5% and 13.4% of the total variation (Fig. 4). Three groups of strongly correlated parameters were detected: (i) global shell size parameters (L_{sh}, W_{sh}, W_{lw}, L_{pw1}, L_{pw2}, L_{lw1}, L_{lw2}), (ii) spire parameters (W_{aw}, W_{pw}, L_{sp1} and L_{sp2}) and (iii) aperture parameters (L_{ap1}, L_{ap2}, W_{ap1}, W_{ap2}). The first two sets of variables, which are linked to global shell size, were primarily associated with PC1. This first axis was thus interpreted as a size factor. The third set of parameters, related to shell aperture, were primarily associated with PC2. The projection of the individuals in the PC1 x PC2 factorial plane revealed that, whereas females and males had similar distribution patterns along PC1, they differed along PC2 (Fig. 4).

The DFA computed with *sex* as the discriminate factor was significant (WL = 0.4241, p = 0.0002). Scores of WL with the probability associated with each variable indicated that the two sexes differed significantly in one aperture parameter (W_{ap2} with WL = 0.501 and $p < 0.008$). The global reclassification score of the discriminating linear functions was 86.44% (78.26% for females and 91.67% for males). These results suggest that females differ from males by having a larger aperture width (W_{ap2}), but not by shell size.

Between populations, the same DFA analyses were then performed on all populations referred, taxonomically and genetically, to the group *utriculus*: 136 specimens were sexed, 62% males and 38% females. This DFA was significant (WL = 0.725, p = 0.0004), but the two sexes differed significantly in the parameter L_{ap1}, another aperture parameter (L_{ap1} with WL = 0.755, p = 0.028).

Finally, a DFA was performed with *sex* as the discriminate factor on the global data set of 358 sexed specimens. The analyses was globally significant (WL = 0.914, p = 0.011), indicating that the set of variables chosen allows discrimination between the sexes. Scores of WL with the probability associated with each variable suggested that the two sexes differed significantly only by one aperture parameter (L_{ap1} with WL = 0.927 and $p < 0.030$).

In order to test for the respective effects of sexual dimorphism and taxonomic differences, two analyses of variance (ANOVA) were performed on the LSR global data set on the two aperture parameters W_{ap2} and L_{ap1} that significantly differed between males and females. First, we tested the effects of the two factors *taxonomic group* and *sex* and their interaction, and then the effect of the factors *genetic group* and *sex* and their interaction. These two ANOVAs revealed that both effects of *taxonomic group* and *genetic group* were significant for the two parameters whereas the effect of *sex* was not significant in any analysis.

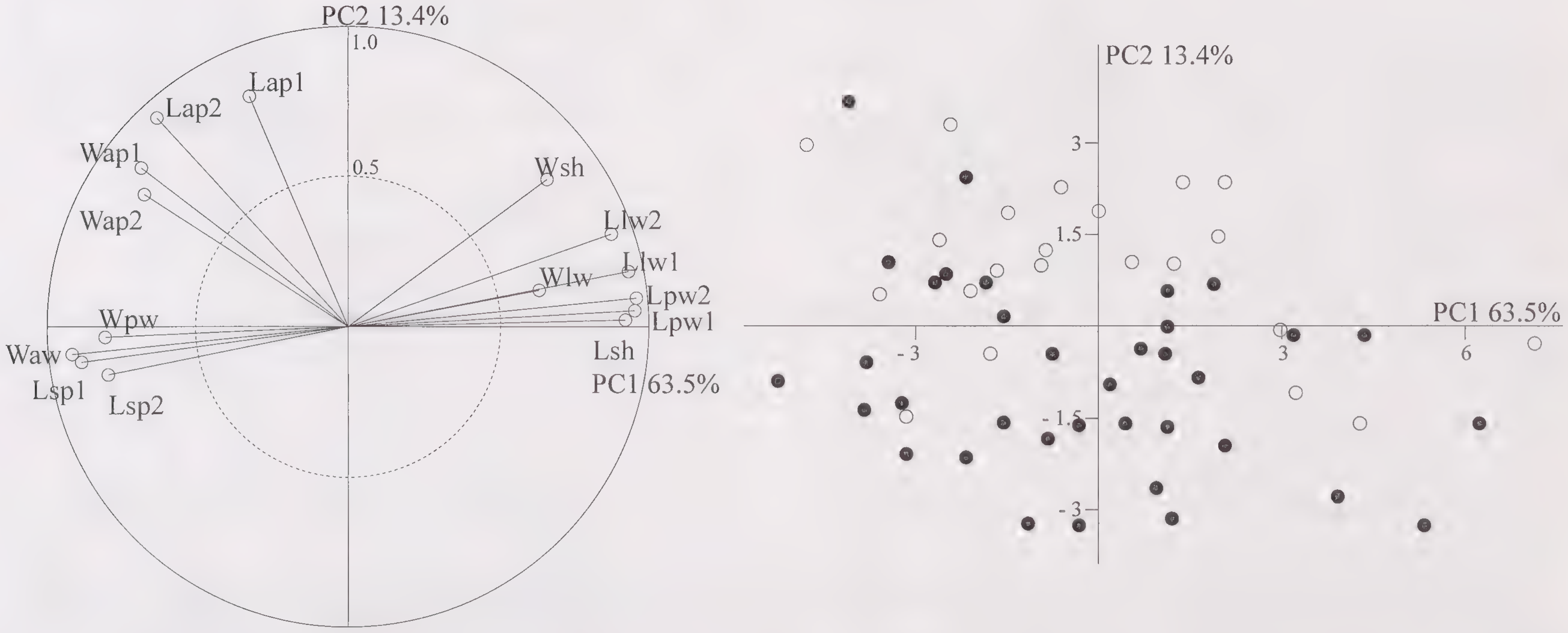

FIG. 4. Sexual dimorphism in Roq population of *Bythinella*: results of PCA. Left Correlation of the 15 parameters measured on the shell to the first two principal components PC1 and PC2. Right Projection of the individuals in the first factorial plan PC1 x PC2. Open circles are females and filled circles are males.

TABLE 5. Correlations matrix between the variables and the first two discriminate functions (F1 and F2) for the two alternative *Bythinella* hypotheses (Taxon group and Genetic group).

	Taxon group hypothesis		Genetic group hypothesis	
Shell parameters	F1	F2	F1	F2
Lsh	-0.027	0.189	-0.058	-0.243
Wsh	-0.460	0.160	-0.495	-0.216
Lap2	0.001	-0.300	0.017	0.327
Wap1	0.076	-0.217	0.103	0.265
Wlw	-0.504	0.084	-0.407	-0.008
Lpw1	-0.068	0.209	-0.102	-0.267
Lpw2	-0.090	0.228	-0.125	-0.289
Llw1	-0.108	0.200	-0.147	-0.267
Llw2	-0.188	0.184	-0.237	-0.264
Waw	0.263	-0.184	0.307	0.256
Wpw	0.166	-0.069	0.207	0.145
Lsp1	0.368	-0.179	0.418	0.256
Lsp2	0.465	-0.147	0.523	0.230
Eingenvalue	3.756	1.383	3.308	1.318
Cum. Eigenv.	53.8%	73.7%	63.3%	88.5%

For each of the two aperture parameters, the *taxonomic group* × *sex* interaction was not significant whereas the *genetic group* × *sex* interaction was significant but only for the W_{ap2} parameter (L_{ap1} F-test = 1.32 with p = 0.267 and W_{ap2} F-test = 4.226 with p = 0.006). For this parameter, the *post hoc* test revealed that the *genetic group* × *sex* interaction was caused

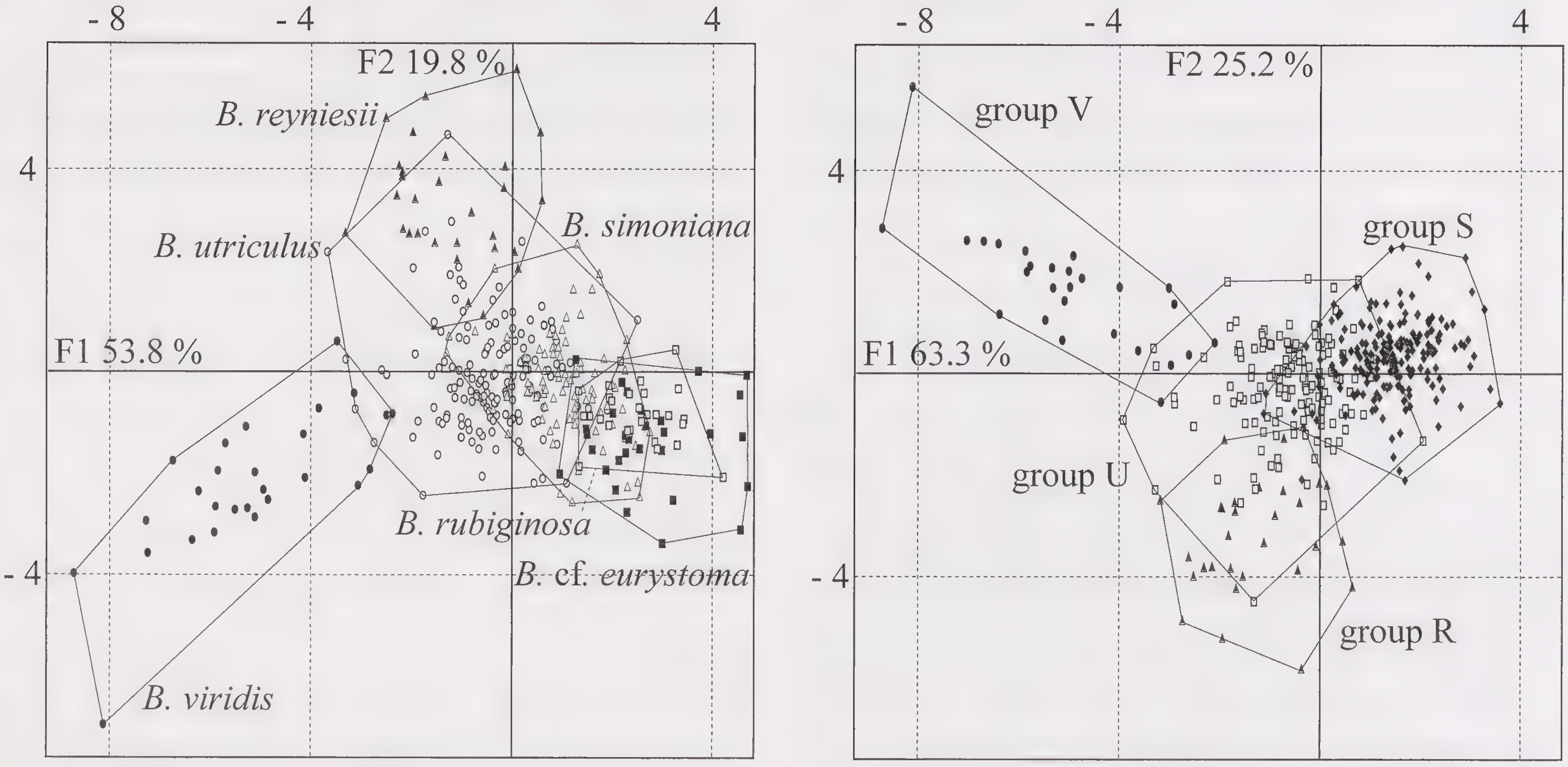

FIG. 5. DFA testing the two alternative hypotheses Taxon groups *vs* Genetic groups within *Bythinella* species. Left Taxon groups hypothesis. Filled circles: *B. viridis* (one population), open circles: *B. utriculus* (four populations), filled triangles: *B.* reyniesii (one population), open triangles: *B. simoniana* (three populations), filled squares: *B.* cf. *eurystoma* (one population), open squares: *B. rubiginosa* (one population). Right Genetic groups hypotheses. Filled circles: group V (*B. viridis*, one population), filled triangles: group R (*B. reyniesii*, one population), open squares: group U (*B. utriculus*, four populations), filled rhombus: group S (*B. simoniana*, *B. rubiginosa*, *B.* cf. *eurystoma* and Aud3 population, total six populations).

TABLE 6. Global reclassification scores of the discriminating linear functions for the two *Bythinella* hypotheses. Each line gives the number of specimens of a given taxon classified as any of the taxa, and the percentage of each that are correctly classified.

	Taxon group hypothesis						
Bythinella	% correct	*viridis*	*reyniesii*	*utriculus*	*simoniana*	cf. *eurystoma*	*rubiginosa*
viridis	88.9%	24	0	3	0	0	0
reyniesii	90.0%	0	27	3	0	0	0
utriculus	87.2%	0	5	129	13	1	0
simoniana	82.2%	0	0	13	74	1	2
cf. *eurystoma*	83.3%	0	0	1	0	25	4
rubiginosa	83.3%	0	0	1	2	2	25
Total	85.6%	24	32	150	89	29	31

	Genetic group hypothesis				
	% correct	Group V	Group R	Group U	Group S
Group V	81.5%	22	0	5	0
Group R	90.0%	0	27	3	0
Group U	86.5%	1	5	128	14
Group S	92.8%	0	0	13	167
Total	89.4%	23	32	149	181

by Por (group R, attributed to *B. reyniesii*), in which the sex ratio was 24% males and 76% females.

To summarize, the analysis shows that variation of aperture parameters is mainly explained by differences between "species" (defined on either the *taxonomic* or *genetic* delimitation hypotheses), and that sexual dimorphism within a population is manifest in aperture size. To minimize the effect of the sexual dimorphism, the two aperture parameters were removed in the subsequent analyses.

Shell Variation Among Populations and Taxa – To explore the pattern of shell variation, a PCA was carried out on the whole *LSR* dataset (385 individuals of both sexes). The first factorial plane accounted for 92.1% of the global variation with 80.3% by PC1 and 11.8% by PC2 (graph not presented here). Three groups of strongly correlated parameters were detected: (i) spire and aperture parameters negatively correlated with the PC1, (ii) height of the various whorls positively correlated with PC1, and (iii) shell width and width of the last whorl negatively correlated with PC2. The projection of the individuals in this factorial plane revealed a strong overlap between the various populations. On the PC2 axis, only individuals of *B. viridis* had a distinct position. Thus, except for this taxon, the parameters used in this analysis did not reveal any differences among groups of individuals when using either the taxonomic or the genetic hypotheses.

Taxon Hypothesis – The DFA on the LSR global data set (less the taxonomically doubtful Aud3 population and L_{ap1} and W_{ap2}) performed with *Taxon* as discriminate factor was significant ($WL = 0.023$, $p < 0.05$). Scores of WL with probability associated with each variable showed that all parameters significantly discriminate the taxonomic groups. Projection of the individuals on the first factorial plane F1 x F2 (each axis respectively accounting for 53.8% and 19.8% of the total variance) allowed discrimination, mainly on the F1 axis, of a cluster of individuals attributed to *B. viridis*. The F1 axis was mainly correlated with spire parameters (W_{pw}, L_{sp1} and L_{sp2}), whereas the F2 axis was mainly explained by a size effect (Table 5). Indeed, for this axis, all variables contributed the same weight and were strongly correlated (Fig. 5). In this F1 x F2 factorial plane, other taxonomic groups were poorly resolved with an extensive overlap among them. For example, individuals referred to *B. simoniana*, *B.* cf. *eurystoma* and *B. rubiginosa* were not separated.

The global reclassification score (Table 6) of the discriminating linear functions was 85.6%, with specific scores ranging from 83.3% (individuals attributed to *B.* cf. *eurystoma and B. rubiginosa*) to 90.0% (individuals attributed to *B. reyniesii*).

Genetic Hypothesis – DFAs performed on the LSR global data set (less L_{ap1} and W_{ap2}) with *Genetic groups* as discriminate factor were also significant (WL = 0.062, $p < 0.05$). Scores of WL with probability associated for each variable showed that all parameters significantly discriminated the genetic groups. The F1 x F2 factorial plan accounted for 88.5% of the global variance, with 63.3% for F1 and 25.2% for F2 (Table 5). The spire parameters are positively correlated with F1, whereas W_{sh} and W_{lw} were negatively correlated with the same axis. The W_{ap1} and L_{ap2} parameters contributed essentially to F2. The projection of individuals in this factorial plane revealed three distinct groups on the F1 axis, corresponding to individuals assigned to groups V, U and S defined below (Fig. 5). On the other hand, F2 allowed separation of group R from all other groups. The global reclassification scores (Table 6) were 89.3% under the *Genetic* hypothesis, with reclassification scores ranging from 81.5% (group V) to 92.8% (group S).

From this result, we can identify four morphogroups based mainly on spire parameters: (i) group V with a small spire and a large last whorl that gives the shell an ovoid outline; (ii) group S with a large overall size and a high spire that gives the shell an elongate outline; (iii) group U, intermediate between V and S, with an outline that can be described as ovoid-elongate; (iv) group R with a spire height equal to the height of the last whorl and a large aperture, that gives the shell a pupoid outline.

DISCUSSION AND CONCLUSION

Our analyses confirmed that all specimens attributed to *B. viridis* (Fig. 6A), which are used as out-group, were morphologically and genetically distinct from all Pyrenean specimens.

Within the Pyrenean sample, two species delimitations resulting from our analysis confirmed earlier traditional species delimitations (Bernasconi, 2000). Specimens attributed to *B. reyniesii* (Fig. 6B) were both morphologically and genetically distinct from all other specimens included in our study. The morphometric analysis describes the shell as having a spire equal to the height of the last whorl and a large aperture, resulting in a pupoid overall shape. However, since a single population was included in the study, this description lacks robustness and we do not know how the characters observed may be subject to phenotypic plasticity or to genetic variability.

The second validated species was *B. utriculus* (Fig. 6F), in which specimens from all populations clustered both as a monophyletic group (DNA sequences) and as a genetic group (isozymes). We hypothesize that the entire clade can be classified as a single species. As several populations are included within this species, our morphometric characterisation integrates at least part of the phenotypic plasticity component of shell shape variability.

All other populations formed a single genetic and phylogenetic group. Morphometric analysis describes the shell within this group as having a large overall size and a high spire that gives it an elongate shape. This group included the nominal *B. simoniana* (Fig. 6D), characterized in traditional taxonomy by axial varices on the last whorl – a character not confirmed in our analysis as of specific diagnostic value – and also included specimens from the two springs complex at Audinac, the cold spring (Fig. 6C) and the thermal spring type locality of *B. rubiginosa* (Fig. 6E), and their confluence. Thus, this cluster groups at least two nominal taxa that should be considered a single species, to be named *B. rubiginosa* based on the Principle of Priority (ICZN *Code* Art. 23.3). The high quantitative (size) or qualitative (varices) shell variability observed in this group may be due either to phenotypic plasticity or to intraspecific genetic variation.

However, although DFA analyses allow separation of these genetic groups, the PCA results highlighted overlaps among most populations, indicating that *a priori* determinations at the species level rank in *Bythinella* are still difficult even when using the shell characters here identified. Moreover, these shell characters cannot be validated for alpha-taxonomy without a robust test at the scale of the genus. We show here that it is possible to give an alternative species delimitation hypothesis to the traditional view in an evolutionary framework and that these newly defined entities exhibit distinct morphological features. We agree with Wilke et al. (2002) about the necessity to use molecular markers and morphometrical approaches in concert to study cryptic or closely related species.

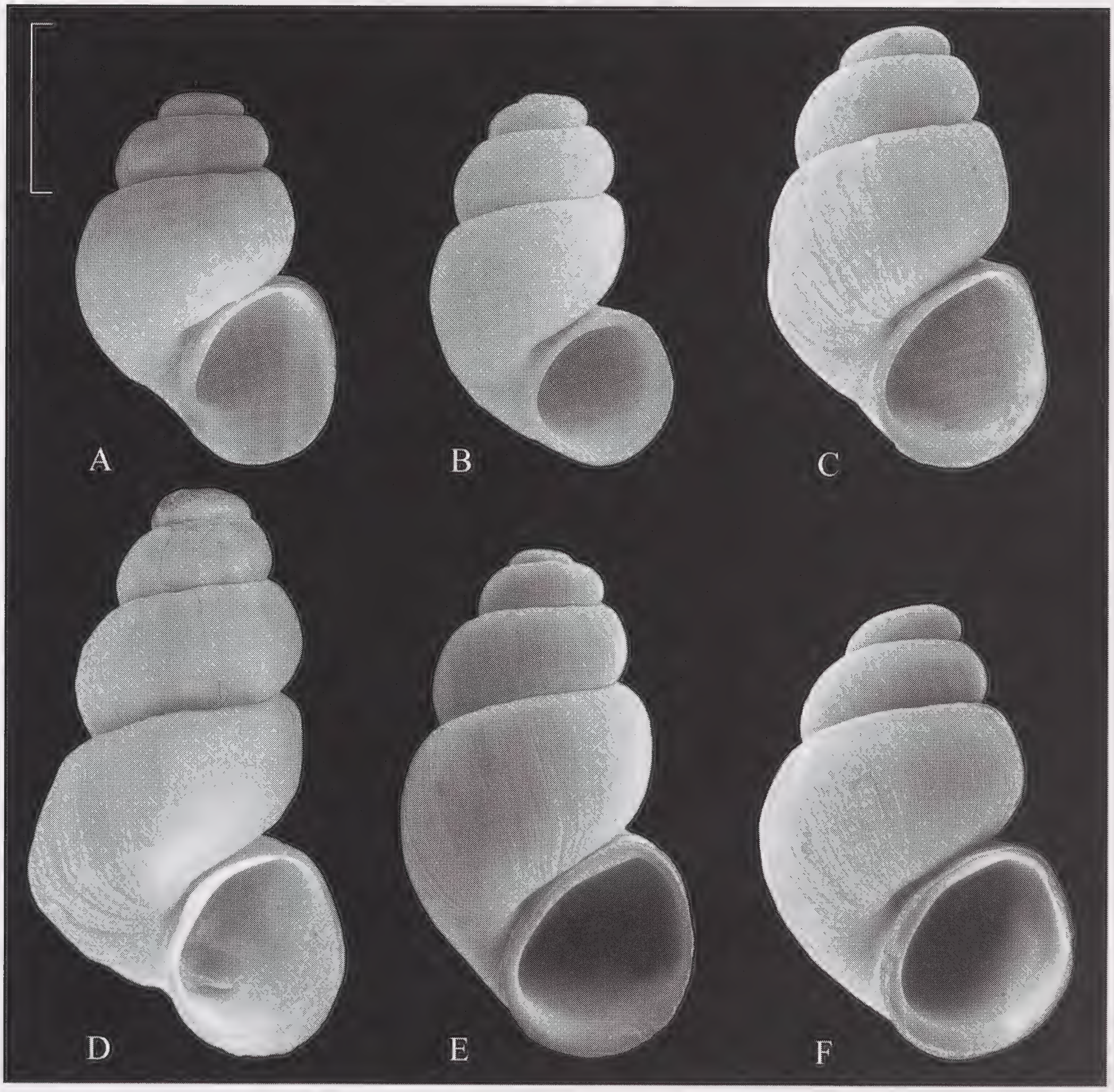

FIG. 6. Shells of the nominal species of *Bythinella* studied. A: *B. viridis* (Che); B: *B. reyniesii* (Por); C: *B.* cf. *eurystoma* (Aud2); D: *B. simoniana* (Eng); E: *B. rubiginosa* (Aud1); F: *B. utriculus* (Roq) (Scale 1 mm).

Genetic studies of Polish populations revealed weak genetic differentiation primarily correlated with geographic distance (Falniowski et al., 1998, 1999). The former suggested that "… the superspecies status of the genus (Giusti & Pezzoli, 1977) seems the most justified". The term superspecies was first used by Mayr (1931) and subsequently defined by Mayr & Ashlock (1991) as "a monophyletic group of closely related and entirely or largely allopatric species that are too distinct to be included in a single species or that demonstrate their reproduction isolation in a zone of contact." Our data don't refute this opinion within the Pyrenean area where a mosaic of populations probably belonging to different species more or less morphologically differentiated seems to coexist in allopatry.

Nevertheless, we show that some shell characters that were traditionally used to segregate species of *Bythinella* (see, for example, Bernasconi, 2000) do not necessarily have taxonomic significance, a result agreeing with that of others working on other hydrobioid groups (Falniowski & Wilke, 2001; Wilke & Falniowski, 2001; Szarowska & Wilke, 2004). The high intraspecific shell variability may be linked to biotic factors, such as food availability, parasite-induced gigantism (Jourdane, 1979; Gorbushin, 1997; Probst & Kube, 1999), or sexual dimorphism (Ponder et al., 1999; Kurata & Kikuchi, 2000; Velecka & Jüttner, 2000; Chiu et al., 2002). Our study corroborates Falniowski's (1987) observation that sexual dimorphism is rather low in *Bythinella*, females differing from males by their generally larger shell aperture. However, as sex ratio appears not fixed in populations of *Bythinella*, superficial morphological differences among populations may also be a consequence of different sex ratios, and contribute to fuzzy species delimitations. Shell variability may also be linked to abiotic parameters, such as temperature (Brown & Richardson, 1992). In the three Audinac populations of *B. rubiginosa*, specimens from the thermal spring are largest, specimens from the cold spring are smallest, and specimens from the confluence are intermediate.

To conclude, the present work offers an example of a three-step integrative taxonomy starting from (i) primary hypotheses (current species delimitations based on phenetic characters), to (ii) the test of these hypotheses by criteria that are conceptually sound. Population genetics approaches allow to identify reproductively isolated groups. Molecular phylogeny allows the recognition of monophyletic groups and thus the detection of groups that share ancestry. These two criteria, derived from the BSC and the PSC, permit new species delimitation, and finally (iii) a feedback to a new taxonomic hypothesis associated with new phenetic descriptors. Therefore, the final description corresponds to the most fitting hypothesis given the data at the time of the description.

A correct delimitation of species has important consequences both for a reliable estimation of biodiversity but also when drawing conservation priorities. Two of the nominal species in the present study (*B. viridis* and *B. reyniesii*) are categorized as Vulnerable in the IUCN Red List and are also protected under French law. Both emerged from the present study as probably distinct evolutionary units.

Our study was restricted to a small part of the geographical range of *Bythinella* including a subset of its nominal species and used a low number of isozyme loci and a short gene fragment. Thus, our results are of preliminary nature. In order to validate them, we need to expand this approach to a global revision of the genus, including supplementary mitochondrial and nuclear markers. However, the challenges to expanding this model are immense. The literature on European hydrobioids is replete with names of unknown significance (Davis, 2004) that blur evaluations of regional species richness, conservation priorities and evolutionary history. This is because many nominal species were established by 19th and 20th century authors that (i) worked on a local or regional basis, and/or (ii) worked outside a context of evolutionary systematics. Their approach to alpha-taxonomy has also occasionally injected in the literature names based on few, sometimes single, specimens only weakly differentiated conchologically. Because the present study shows that genetic or phylogenetic distinctiveness cannot be inferred from the degree of shell distinctiveness, the challenge to a comprehensive revision of *Bythinella* is that these nominal species have to be re-evaluated based on populations sampled alive from their type locality. At this moment, we do not know whether an integrative revision of *Bythinella* will result in just a few or several dozen species.

How do the present results impact the systematics of fossil *Bythinella*, and does the result of the present study preclude naming any new *Bythinella* species based on shell characters only? Despite the numerous nominal species of *Bythinella* described by 19th and 20th century authors, new exploration of little-known aquifers, in particular hypogean environments in southern France, still leads to the discovery of new morphotypes. Sometimes, only empty shells are known and living specimens escape collection despite intensive searches. We understand that it may be necessary, for communication or conservation purposes, to name such taxa; however, it is essential that the primary description is based on shell characters that have been tested for their robustness regarding species delimitation.

Stability in the alpha-taxonomy of hydrobioids will most likely be reached through an integrative taxonomy approach (Sites & Marshall, 2003, 2004). A possible approach would be the Hennigian inter-nodal species concept derived from the Theory of Evolution (Samadi & Barberousse, 2006) that here proved an efficient tool in the study of Pyrenean *Bythinella*.

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LAND SNAIL DIVERSITY OF THE SAVANNA/FOREST MOSAIC IN LOPÉ NATIONAL PARK, GABON

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ABSTRACT

We present the assessment of the land snail diversity in approximately 50 km² of savanna/forest mosaic in the northern part of Lopé National Park, Gabon, taking into account habitat variation and seasons. A total of 3,745 specimens were collected, yielding 74 species from 12 families, with Subulinidae being the most speciose family. Most specimens were not identified but assigned to Recognizable Taxonomic Units. Extrapolations suggest that the true diversity of the area lies between 79 and 132 species. Overall snail abundance was low, and most species were minute. Spatial and habitat heterogeneity was high, with 33.8% of the species collected from one station only. Rare species made up a considerable proportion of the fauna, with 23.0% of the species represented by one specimen only. The most species-rich habitats were mature forest, Marantaceae forest, rocky forest, and forest fragments isolated in savanna, in that order. Savanna was the least species-rich habitat, and no species were confined to this habitat. Benefits and drawbacks of the Recognizable Taxonomic Units approach are discussed, and suggestions for maximizing mollusc inventories in tropical forests are proposed.

Key words: Gabon, Mollusca, land-snail, biodiversity, Recognizable Taxonomic Unit, rainforest, rarity.

INTRODUCTION

Tropical rain forests are disappearing or being degraded at an alarming rate all over the world, and African forests have been reduced to one third of their original extent (Sayer et al., 1992). West Central Africa has the largest remaining block of forest, and Gabon retains the highest percentage of forest cover (between 87% and 96% depending on the estimates). However, these forests are now targets for logging companies, and the annual rate of deforestation for Central Africa is 0.6% (Sayer et al., 1992). In Gabon, 68% of the original extent of frontier forests (large, ecologically intact and relatively undisturbed natural forests; Bryant et al., 1997) has been lost, 100% of the remainder is threatened, and the frontier forest index is 68, on a scale from 0 to 99, 99 being the worst possible score (Bryant et al., 1997). Large tracts of forest have disappeared before information on their ecology and biodiversity could be obtained. For sound and reliable conservation actions to be undertaken, it is important to document the diversity within the remaining forests.

In contrast to insect diversity, the mollusc fauna of tropical forests was until recently believed to be relatively depauperate (Solem, 1984). Indeed, the lack of calcium in the soils of the Congo-Zaire and Amazon basins argues against expectations of a rich and diverse malacofauna. Recent studies (Emberton et al., 1996; Tattersfield, 1996; De Winter & Gittenberger, 1998; Gargominy & Ripken, 1998; Schilthuizen & Rutjes, 2001; Seddon et al., 2005) used intensive sampling methods, including litter sieving, and have shown that previous assumptions, at least in part, were wrong: for example, in Cameroon, 97 gastropod species were found in 1 km² of apparently homogeneous forest (De Winter & Gittenberger, 1998); in French Guiana, up to 19 land snail species were found in only 1 m² of forest floor (Gargominy & Ripken, 1998). As most of the species are minute, rare, or hard to find (in these studies, fewer than five individuals were found for many species), the level of diversity is hard to detect. From the little information available (Rogers & Homewood, 1982; De Winter, 1995), it seems that at least some species have a restricted geographical range, which is important from a conservation perspective. Solem (1984) predicted a median range of less than 100 km for all land snail species, and probably less than 50 km, but on the other hand gave a median range of 0.825 km² for 28 species of camaenid land snails in the Kimberley Range (Western Australia) (Solem, 1988a)! His preliminary speculations on generally small range size and high allopatric diversity have been confirmed by subsequent works (reviewed in Seddon et al., 2005).

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The general paucity of studies concerning tropical malacofaunas is partly because taxonomists, for whom conservation is not always the first priority, are often reluctant to publish the results of a study before their material is fully identified/described. The lack of taxonomic expertise on molluscs represents a bottleneck (the taxonomic impediment, see the website of the Global Taxonomy Initiative at http://www.biodiv.org/programmes/cross-cutting/taxonomy/), emphasized by the fact that there are few comprehensive collections in museums, and that published knowledge is scattered and very limited especially in regard to more recent literature. Notably, there are no checklists for West and Central Africa, and very few for East Africa (Verdcourt, 1983; Bruggen, 1993). As a result, tropical mollusc faunas are poorly known: for instance, there is only a single recent paper (De Winter, 1995) for Gabon, the situation being similar for Congo, Cameroon, and Equatorial Guinea. The main reference we used for identifications during our study dates from 1919 and concerns the Democratic Republic of Congo (Pilsbry, 1919). For West and West-Central Africa, there are altogether approximately 500 papers on molluscs (De Winter, pers. comm.). The contrast with the situation in European countries is striking: for example, Falkner et al. (2002) present a list of 377 references published between 1990 and 1999 dealing with the systematics and distribution of the malacofauna of France alone, besides those on anatomy or biology; for the same period, the Zoological Record online lists only 15 papers on these topics for Gabon, Cameroon, Equatorial Guinea, and Congo combined.

The northern part of the Lopé National Park is an area of forest-savanna mosaic with a relatively high diversity in the studied groups: for example, approximately 1,400 plant species (White & Abernethy, 1997) and 399 species of birds (Christy, pers. comm.) have been recorded in the area. This high diversity is linked to the high diversity in habitats, from open grassland to dense forest, resulting from the recolonisation of savannas by forest since 1500 B.P., and the impact of human-induced fires in savannas (detailed by White, 2000). The area has had protected status since 1946 and was upgraded from Faunal Reserve to National Park in 2002. Field research has been ongoing since 1983, primarily on primates, but also in other areas, including vegetation history, archaeology, and large mammal populations.

The present paper is the result of a conservation-oriented study of the land snail fauna of central Gabon. We document here the molluscan diversity of the northern part of the Lopé National Park and examine its relationship to habitat variation; a separate paper will compare the faunas inside and outside the park (Fontaine et al., in press).

METHODS

Geographical Setting

The study site is the north-eastern part of the Lopé National Park in central Gabon (Fig. 1). Most of the park is covered by semi-deciduous lowland tropical rainforest, with approximately 300 km² of savanna and forest-savanna mosaic along the northern and part of the eastern limits of the park. In our study area, the forest was selectively logged at low intensity (1–2 trees.ha^{-1}) more than 30 years ago, mostly for okoume *Aucoumea klaineana* (Burseraceae) (White et al., 1995). Two major rivers, the Ogooué and the Offoué, as well as many tributaries, run through the area.

The climate is characterised by a well-defined dry season of about three months between June and September, but its beginning and duration vary among years. There is usually a less pronounced and short dry season in January–February. The mean annual rainfall is 1,548 mm, and temperatures vary little

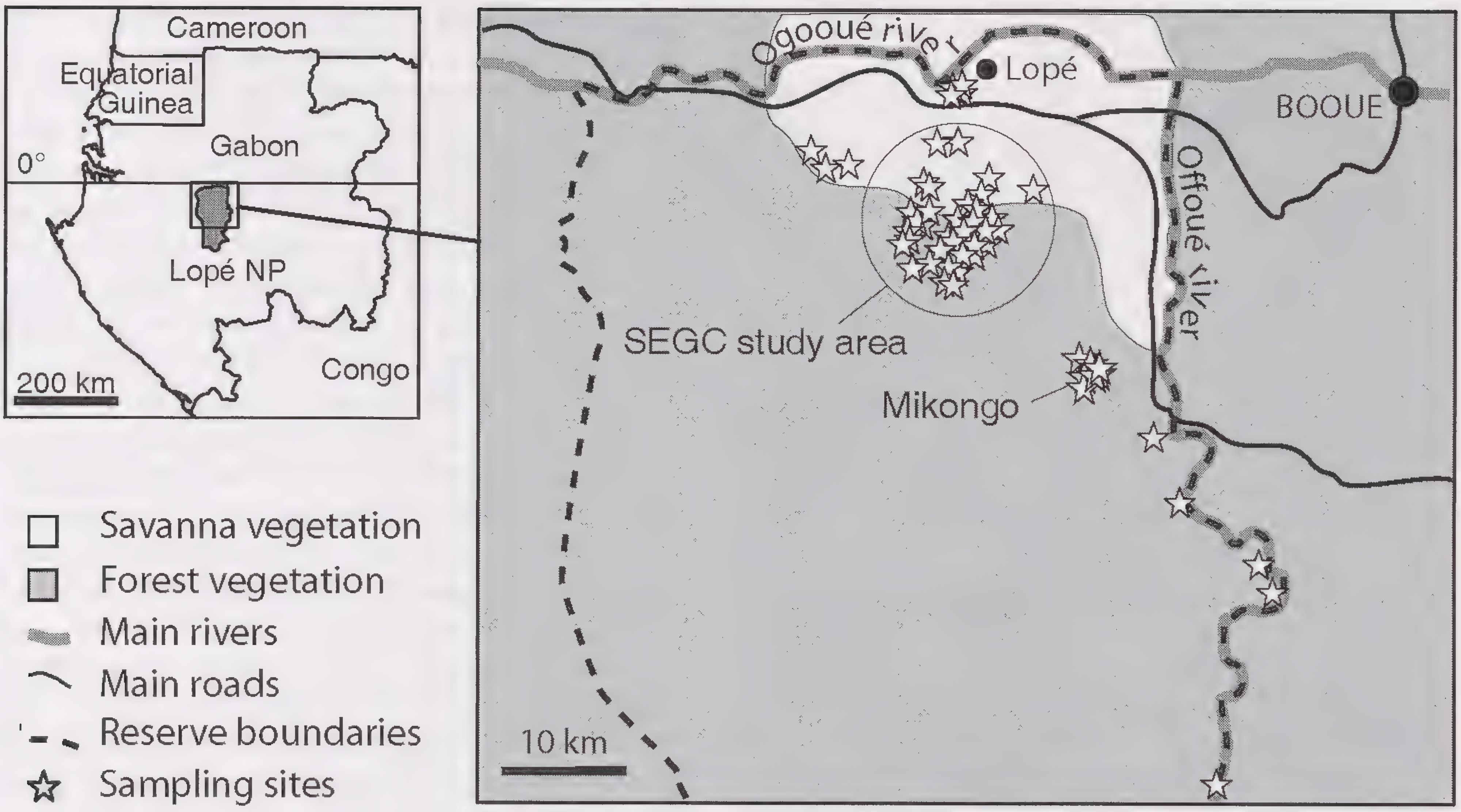

FIG. 1. Location of the study site in Central Gabon, and sampling area of this study.

but are lowest in the dry season; mean monthly maxima vary from 26.8 to 30.8°C and minima from 20.5 to 22.3°C (Tutin et al., 1997).

The geology of the park is dominated by old metamorphic and granitic bedrocks (Nicklès, 1952). In most of the study area, the altitude varies between 200 m and 300 m, with a few hills reaching 700 m.

Twenty forest types have been identified in the Lopé National Park, according to vegetation structure and composition (White, 1992). For the purpose of sampling, we distinguished the following habitats (White et al., 1995; White & Abernethy, 1997):

- Savanna: vegetation dominated by grass with scattered shrubs, mostly *Nauclea latifolia* and *Crossopteryx febrifuga* (Rubiaceae).
- Colonised savannas: adjacent to savannas, resulting from the colonisation of savannas by shrubs and pioneer trees such as *Aucoumea klaineana*, *Lophira alata* (Ochnaceae), and *Sacoglottis gabonensis* (Humiriaceae). As tree cover increases, colonised savannas turn into okoume forest.
- Okoume forest: monodominant *Aucoumea klaineana* forest. Canopy cover is discontinuous at 30–70%.
- Marantaceae forest: dominant tree species are *Aucoumea klaineana* and *Cola lizae* (Sterculiaceae), with other species belonging to the families Annonaceae, Ebenaceae, Mimosaceae, and Myristicaceae relatively common. Canopy cover is discontinuous at about 85%, and the understorey is very dense, consisting primarily of herbaceous Marantaceae and Zingiberaceae.
- Mixed/Mature forest: higher tree species diversity, increased canopy cover (95%) and open understorey. Dominant tree families are Caesalpiniaceae, Olacaceae, Myristicaceae, Sapotaceae, Burseraceae, Irvingiaceae, and Euphorbiaceae.

These vegetation types form a dynamic succession from savanna to mature forest, each type being replaced by the next, to eventually reach the climax of mature forest (White, 2000).

In addition to this succession, other vegetation types were distinguished in our study:

- Rocky forest: associated with rock outcrops and thin soil. Trees rarely exceed 30 cm diameter at breast height; the canopy is 10–20 m high.
- Galleries: forest vegetation along streams and rivers (Ogooué) in savannas.
- Forest fragments: small patches of forest surrounded by savanna. They usually have an anthropogenic origin, being the remains of Iron Age villages (Oslisly & White, 1996). Typical tree families in forest fragments are Moraceae (figs), Arecaceae (palms), Bombacaceae, and Annonaceae.

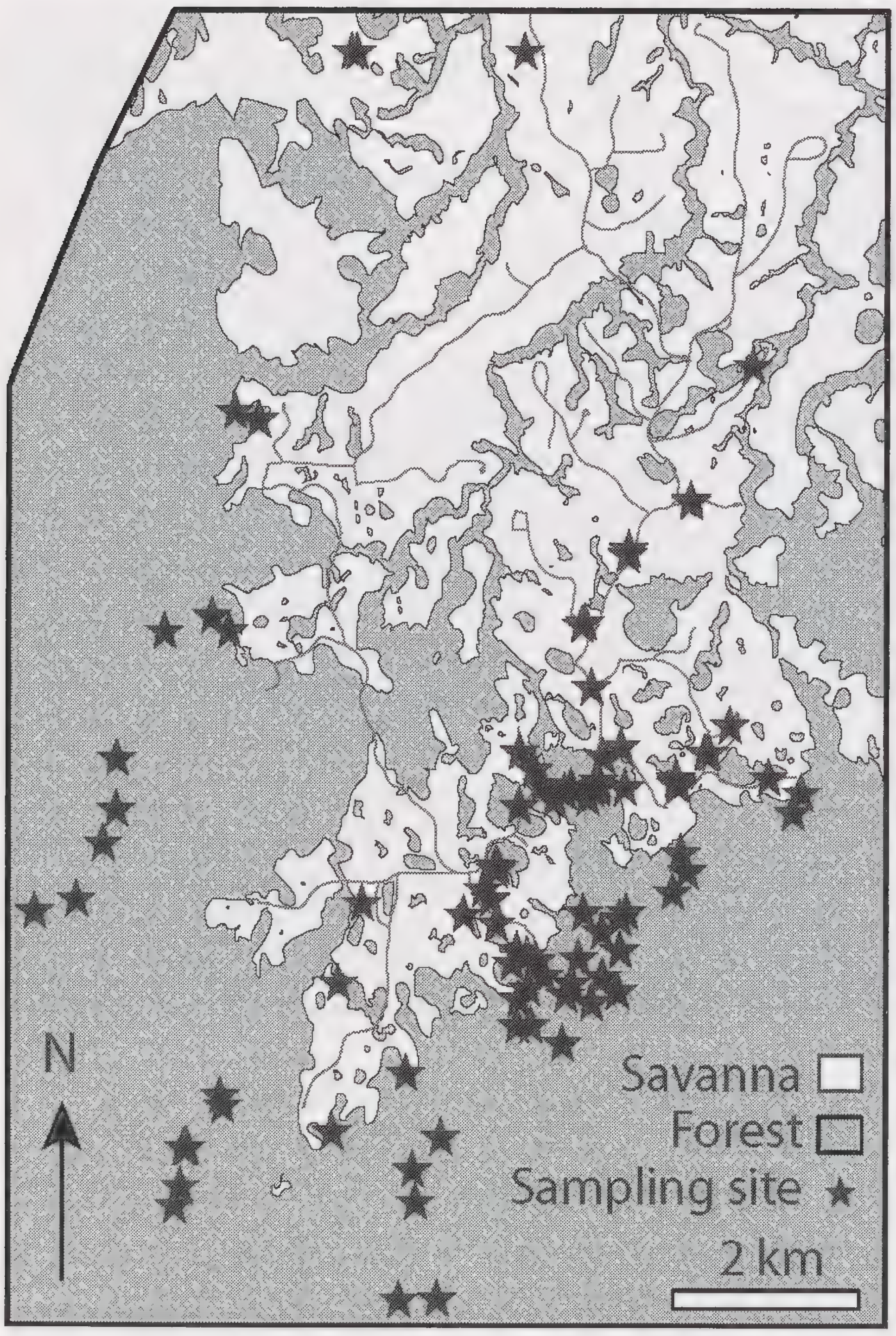

FIG. 2. Location of the sampling stations in the study area of SEGC (38 stations were outside the SEGC study area).

Two other habitats were sampled:

- Anthropogenic: In and around houses. However, we did not sample in plantations around Lopé, a village 15 km from our study site.
- River drifts: flood deposits along rivers. These do not represent a true habitat, because the empty shells they contain have been washed from upstream vegetation types that may be different from the one immediately surrounding the river drift. They were sampled for informative purpose only, but are not included hereafter.

Collecting Effort

Altogether, 132 stations were sampled. The core area surveyed (96 stations) covers approximately 50 km², corresponding to the study site of the Station d'Etude des Gorilles et des Chimpanzés (SEGC) (Fig. 2). We also collected outside the study site of SEGC (38 stations) in scattered places in the northeast of the park, mainly in the Mikongo area (14 stations) and along the Offoué River (eight stations). A station is defined as a collecting locality, spread over 5–10 m² at most, in a single habitat and microhabitat. When two microhabitats were sampled in the vicinity of each other, they were considered as two different stations; this approach differing from most other works (Tattersfield, 1996; De Winter & Gittenberger, 1998; Emberton et al., 1999) in which snails are usually searched for in all microhabitats in quadrats of a given area. This was intended to maximize the efficiency of sampling, focusing on microhabitats rather than habitats. The main microhabitats sampled, representing 89.1% of the stations, were leaf-litter in depressions on the ground (36.5%), leaf-litter between buttresses of large trees (26.3%), rotten logs (12.4%), leaf-litter at the base of trees without buttresses (8.8%), and rock crevices (5.1%). Other microhabitats included house walls, elephant bones, tree trunks, and bare ground. The distribution of stations across habitats and microhabitats is presented Table 1.

Sampling took place in three different periods: 30 August to 7 October 1999 (transition between dry and rainy seasons), 19 June to 11 August 2000 (dry season), and 21 April to 7 June 2001 (rainy season).

For each station, we recorded the geographical coordinates using a GPS GARMIN 12CX, as well as the habitat, microhabitat, exposure, altitude as given by the GPS and date.

At each station, two people spent 30 minutes searching at ground level for live snails, then leaf-litter and a few millimeters of topsoil were collected. This sample was processed at the collecting location with a Winkler sieve (1 cm mesh), the coarse material being checked for shells (empty shells and live animals) and discarded. The remaining material was bagged and sun-dried as soon as possible. The molluscs collected alive were drowned overnight and fixed in 70% ethanol for future dissection.

Once dried, the bagged leaf-litter material was weighed and its volume measured. Altogether, 445 liters of litter were collected. It was passed through 5 mm, 2 mm, and 0.6 mm sieves. The two larger fractions were thoroughly searched with the naked eye, the third one sorted under a dissecting microscope. Material passing through the 0.6 mm sieve was searched for the first three sites, but as it contained no molluscs, as was the case in earlier studies (e.g., Tattersfield, 1996; De Winter & Gittenberger, 1998), it was subsequently discarded.

TABLE 1. Number of stations per microhabitats and habitats.

	Rotten logs	Buttresses	Leaf-litter	Tree base	Tree trunk	Rock crevices	Bare ground	House wall	Elephant bones	Total
Mature	5	14	8	5		1				33
Marantaceae	7	8	8		1	1	3			28
Okoume	1	2	3			1				7
Rocky	2	3	7	1		1				14
F. fragments		8	7	3						18
Gallery	1	1	3	1						5
Col. Savannas	1		4							5
Savannas			6	2		3	1			12
Anthropic								4	1	5
River drifts			5							5

For seven stations, the dry material between 2 mm and 0.6 mm was separated into two equal parts. One was completely searched for molluscs, the other was poured into a bucket of water, and only the floating fraction (once re-dried) was searched for molluscs. As the number of shells found by flotation was not significantly different from that found by complete searching (Wilcoxon matched pairs test: z = 1.57, N = 7, ns), it was assumed that only a negligible number of shells were lost with this method, and the remaining samples were searched after floating, which improved efficiency. This method has been successfully tested by other authors (e.g., Cameron, 1986).

Twelve stations, in various vegetation types, were sampled twice, once during the rainy season (April–May) and once at the beginning of the dry season (end of June) to check for seasonal variability. These stations were marked with metal disks nailed to the trees to allow for their accurate relocation.

Taxonomic Processing and Data Analysis

All specimens were sorted to morphospecies, or Recognizable Taxonomic Units (New, 1999), by an experienced taxonomist (E.N.) according to shell characters, assigned to a family and, when possible, to a described genus or species. Few RTUs received specific identification. As we did not dissect animals, closely related species with similar shells may have been overlooked (in particular, urocyclid semi-slugs), so our diversity results could be underestimates. However, most of our RTUs are equivalent to species as generally understood by mollusc taxonomists, and in the Results and Discussion sections, "RTUs" and "species" refer to the same concept. The genus and, to some extent, family allocations we have used are tentative, and many RTUs currently assigned to the same genus (or family) based on shell characters might belong to different genera (or families). In other words, our results are repeatable at species level, but should not be used to compare genus or family diversity in another country/continent.

In our analyses, we have combined animals collected alive and those collected dead, for two reasons: (a) we collected more dead shells than live animals, and did not want to exclude the bulk of our data from the analyses, and (b) it was sometimes difficult to determine whether shells had been collected alive or dead, because the material was processed by sun-drying, sometimes long after collection.

When possible, juvenile specimens were assigned to a RTU for which we had adult specimens. If more than one RTU matched with the juveniles (mostly urocyclids and some streptaxids), these juveniles were discarded from the diversity and abundance analysis. If the juvenile did not match any of the adult shells, it was treated as a separate RTU.

Heterogeneity between habitats and stations was measured with Whittaker's index I, which is the total number of species recorded (S) divided by the mean number of species per station (Cameron, 1992; De Winter & Gittenberger, 1998). If I equals 1, all the stations have identical faunas, whereas higher values indicate increasing differentiation. Within-habitat evenness was measured by inverse Simpson's index, which provides a good estimate of diversity at relatively small sample sizes and ranks assemblages consistently (Magurran, 2004). With inverse Simpson's index, a higher diversity is reflected by a higher index.

Both height (H) and diameter (D) of the shell were measured from randomly selected adult specimens. As the global pattern of distribution of height and diameter classes is tightly linked to the choice of size classes (Allsopp, 1997; De Winter & Gittenberger, 1998; Bouchet et al., 2002), size classes were first represented with intervals equivalent to a 2-fold transformation. The X-axis was then logarithm (base 2) scaled to the size concerned. In order to get a better representation, we divided each class by 2, that is, using a 2-square-root-fold transformation.

In order to assess faunal assemblages, a Principal Components Analysis and a Cluster Analysis (euclidean distances, Ward's method) were performed using Statistica 6.0 (StatSoft, Inc.).

The authority for the higher classification of Mollusca used in this study is Bouchet & Rocroi (2005).

Voucher material is deposited in the Muséum National d'Histoire Naturelle, Paris (France).

RESULTS

Species Richness

A total of 3,745 specimens representing 74 species were collected at the study site (Appendix). The fauna consists of 12 families (Fig. 3). Subulinidae were the most speciose family, with 26 species (ca. 35% of the total, and 62% of the total number of specimens), followed by Streptaxidae (19 species, 26%) and Urocyclidae (12 species, 16%).

The species accumulation curve (Fig. 4) and the various richness estimators calculated via EstimateS 7.5 (Colwell, 2005) show that our sample represents between 56% (Chao2, 132 species) and 94% (Michaelis-Menton equation, 79 species) of the total extrapolated richness. EstimateS 7.5 provides 95% confidence intervals for Chao1 and Chao2 estimators, which are as follow: Chao1: 82.56–171.54; Chao2: 92.84–250.07.

Shell Size and Shape

The Appendix gives the height and diameter of 71 species collected – Veronicellidae (no shell) and semi-slugs (shells not identifiable to species) were not measured. Major shell dimension (either H or D, whichever is greater) ranged from 1.3 to 118.6 mm with a mean of 10.0 mm. As a result of the high number of small species compared to large ones, the median is only 5.5 mm and the mode is 3.5 mm. Figure 5 gives the distribution of the major shell dimension. Despite different size classes having been used by different authors, the size distribution in our study seems to follow the same pattern as others (Allsopp, 1997; De Winter & Gittenberger, 1998), even in a marine habitat (Bouchet et al., 2002), that is, a Poisson-like distribution with two main peaks, the first (smaller sizes) being higher than the second (larger sizes). Major shell dimension (H or D) of 30 species (42%) is less than 5 mm (37% in Cameroon, De Winter & Gittenberger, 1998); 42 species (59%) have a

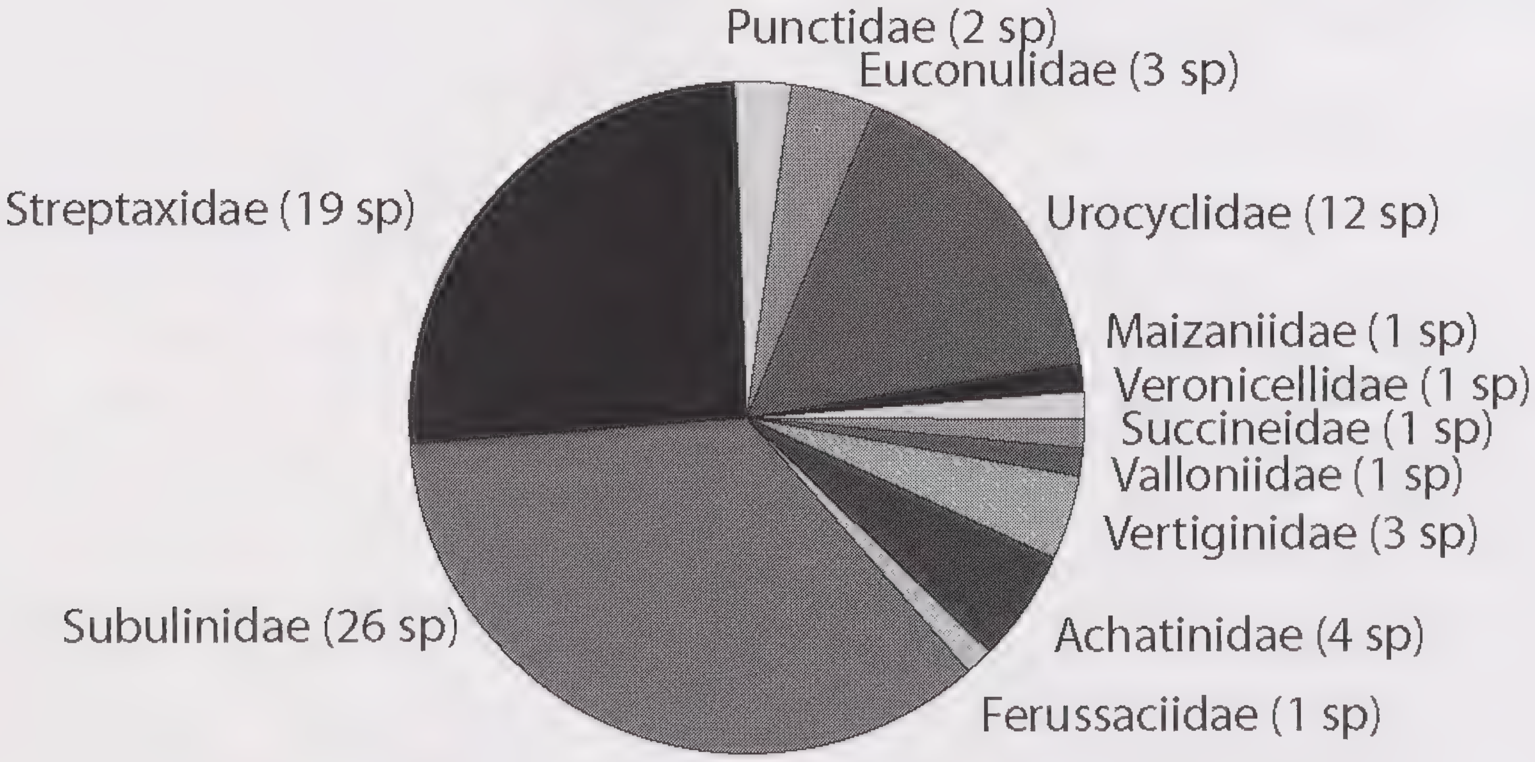

FIG. 3. Distribution of land snail families in Lopé National Park, Gabon.

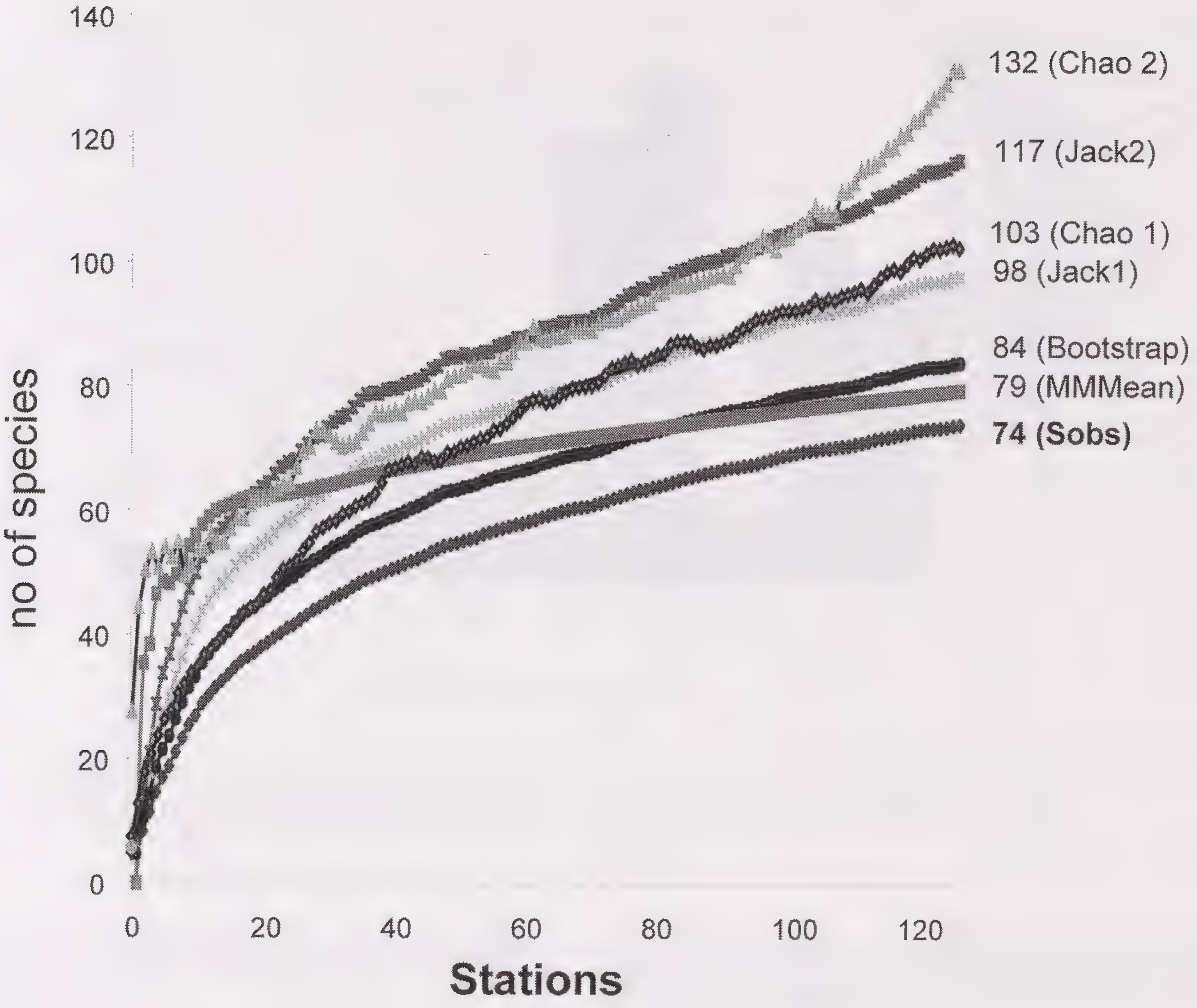

FIG. 4. Species accumulation curves based on EstimateS 7.5 (Colwell, 2005), with various richness estimators.

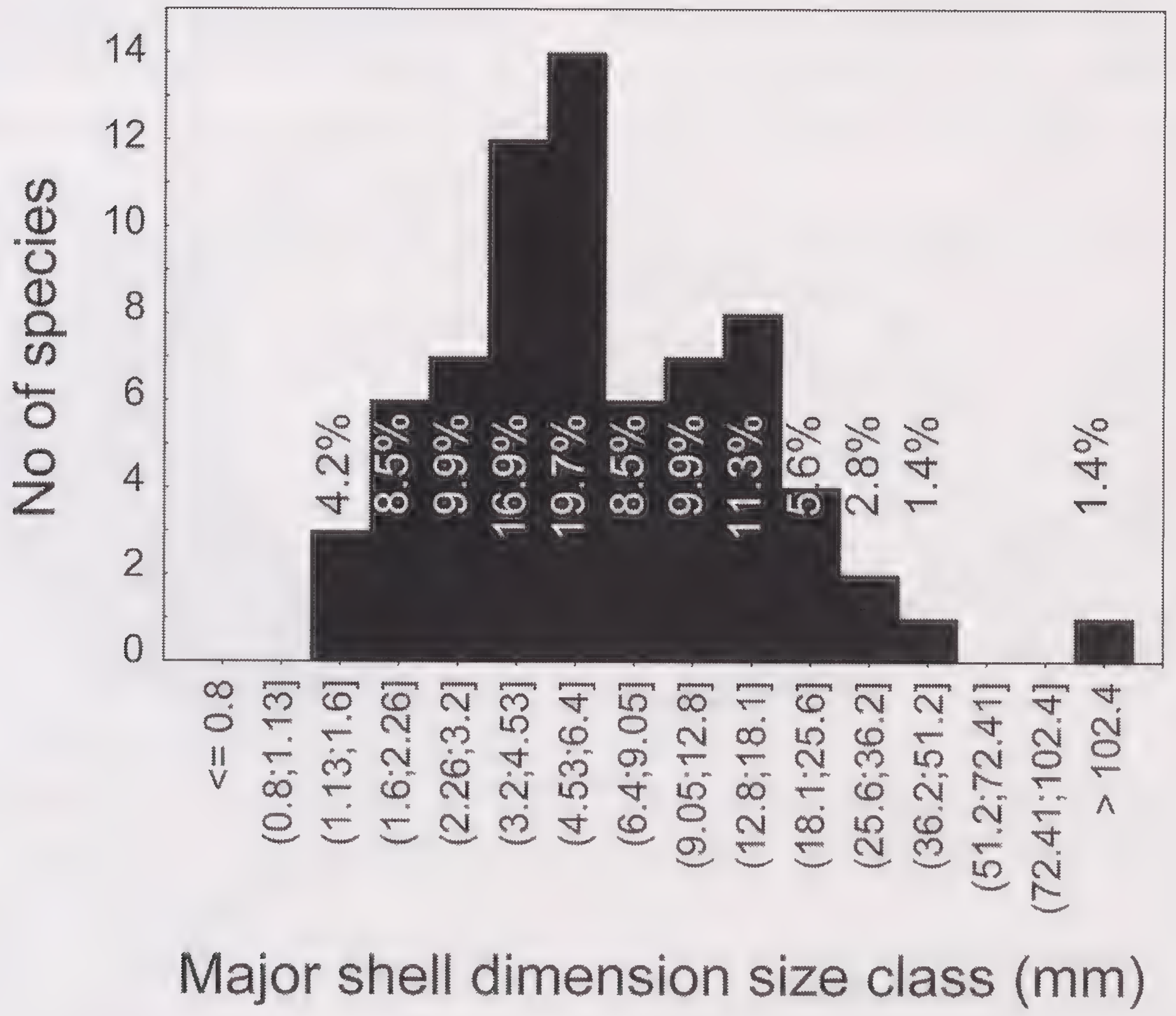

FIG. 5. Major shell dimension (height or diameter) distribution of 71 species living in the Lopé National Park, Gabon, based on randomly selected adult specimens. Size classes are intervals equivalent to a 2-square-root-fold transformation, or a 2-fold transformation for 2 classes.

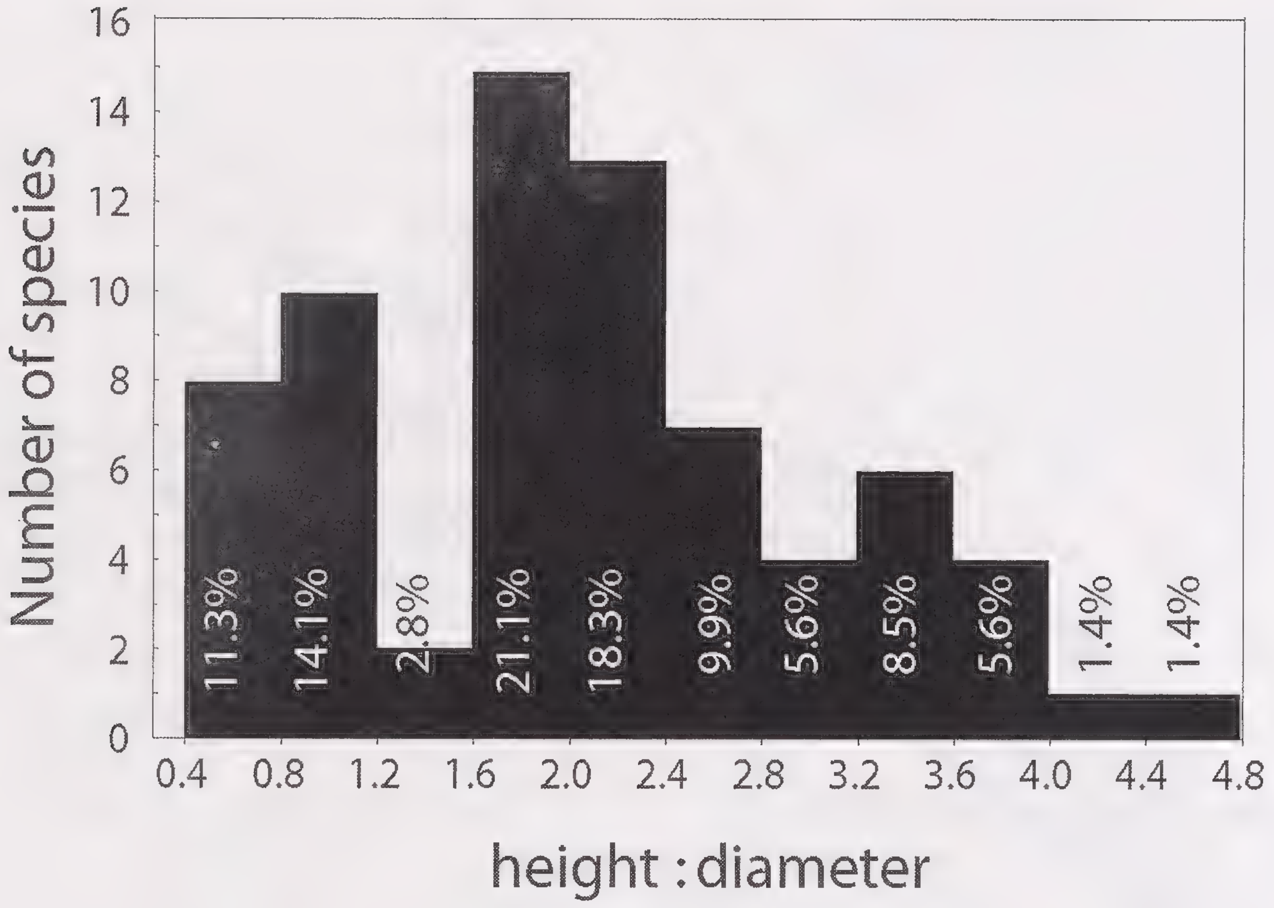

FIG. 6. Shell height:diameter ratio of 71 species living in the Lopé National Park, Gabon, based on randomly selected adult specimens.

major shell dimension less than 6.4 mm, and an additional 25 (35%) are between 6.4 and 25.6 mm.

Figure 6 shows a bimodal distribution of shell height:diameter ratio for the 71 species measured, with a high number of globose to flat species (0.4 < H/D<1.2) and tall (H/D > 1.6) species. No species have H/D < 0.4 and very few have H/D > 4.0.

Rarity

Snail abundance is generally low in the study area. This can be expressed as biological rarity and ecological rarity, *sensu* Bouchet et al. (2002).

Biological Rarity – Biological rarity is estimated by the total number of specimens found of a given species. The dominant feature of

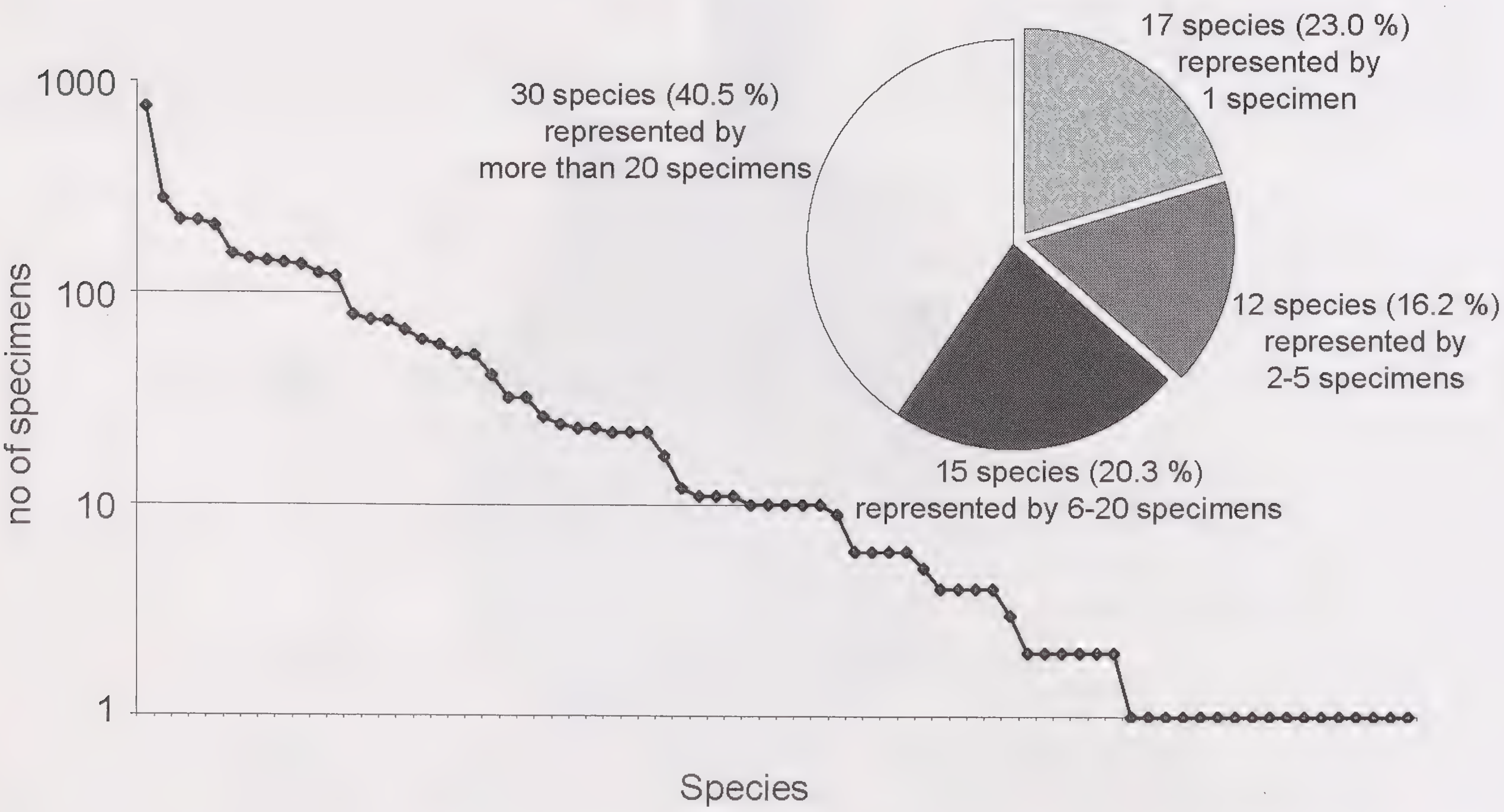

FIG. 7. Biological rarity of the terrestrial molluscs living in the Lopé National Park, Gabon. Proportion of species in four arbitrary abundance (number of specimens) categories and rank-abundance relationship.

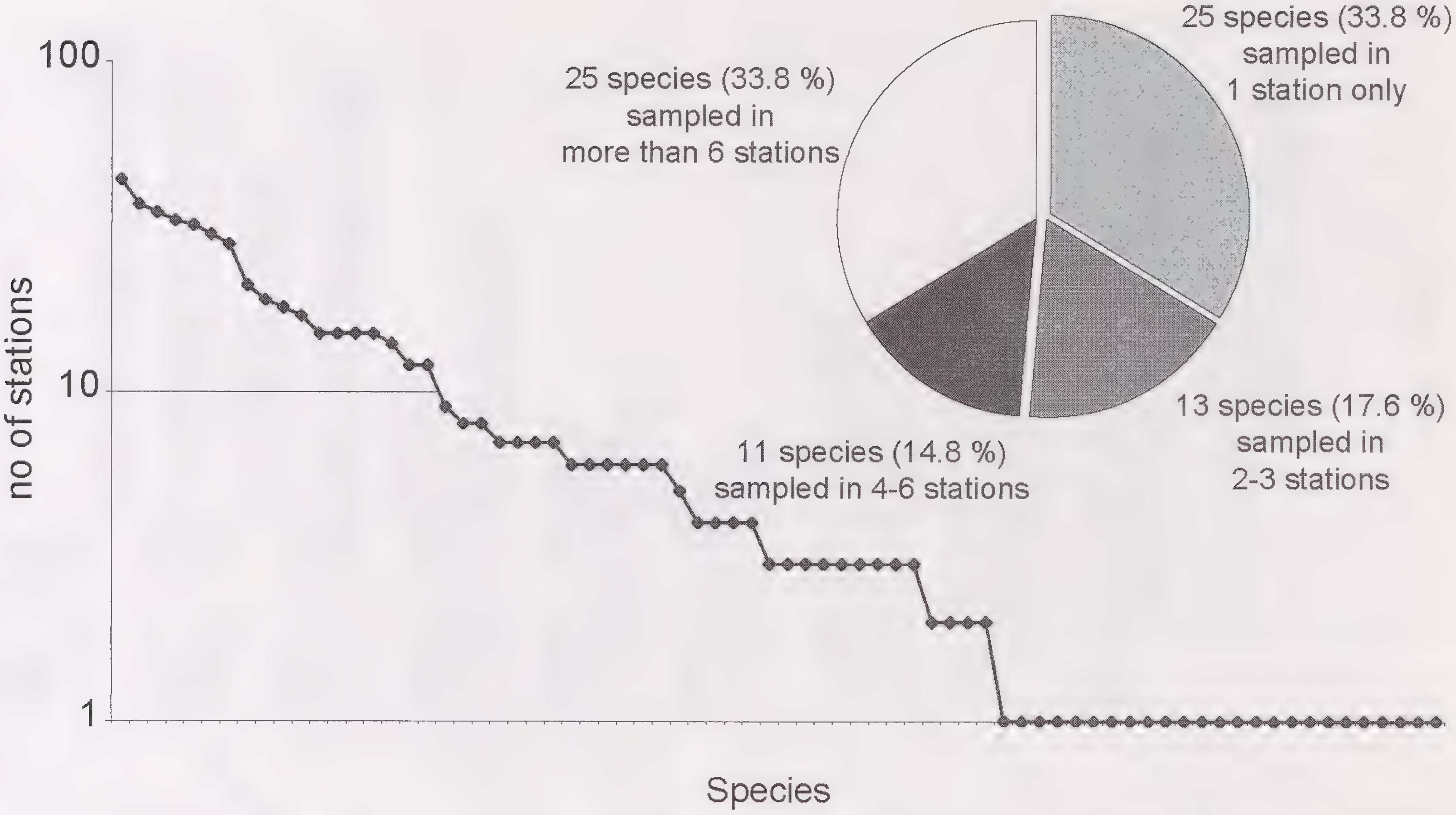

FIG. 8. Ecological rarity of the terrestrial molluscs living in the Lopé National Park, Gabon. Proportion of species in four arbitrary abundance (number of stations of occurrence) categories and rank-abundance relationship.

the fauna is the high frequency of rare species (Fig. 7). The average is 47.4 specimens per species, the median is 10 specimens per species, and the mode is one specimen per species.

A remarkable feature is the relative abundance of one species of Subulinidae, *Subulona decollata* (Morelet, 1873), with 804 specimens, that is, 21.5% of the total number of specimens. It was most abundant at one station (Gab159, in a forest fragment, with 602 specimens), where many individuals were associated with (perhaps feeding on) fallen flowers of *Ceiba pentandra*. Even if this station is discarded, *S. decollata* remains among the most abundant species.

When the quartile definition of rarity is followed (Gaston, 1994), 19 species fall into the category "rare", with no more than two specimens. These species are represented by an average of 1.11 specimens. Among these 19 species, eight are Streptaxidae.

Ecological Rarity – Ecological rarity is estimated by the number of stations at which a species occurred. In Lopé, 25 species (33.8%) were only found at one station (including 11 Streptaxidae, five Subulinidae, four Urocyclidae). These necessarily include the 17 that are represented by only one specimen, plus eight others that are represented by more than one specimen. Twenty-five species (33.8%) were found in more than six stations (Fig. 8). The average frequency of occurrence for the 74 species collected is 8.38 stations per species, the median is four stations per species and the mode is one station per species.

Species Richness and Abundance in Various Habitats

For the 74 species collected, Whittaker's index was 14.0, which indicates a substantial degree of beta diversity. Table 2 gives Simpson's index calculated for various habitats (more than ten sampling stations): according to this index, mature forest, rocky forest and Marantaceae forest are the most diverse habitats.

TABLE 2. Simpson's Inverse Diversity Index for habitats covered by more than 10 stations.

Habitat	Simpson's Index
Mature forest	18.66
Rocky forest	15.19
Marantaceae forest	14.19
Forest fragments	4.54
Savanna	4.48

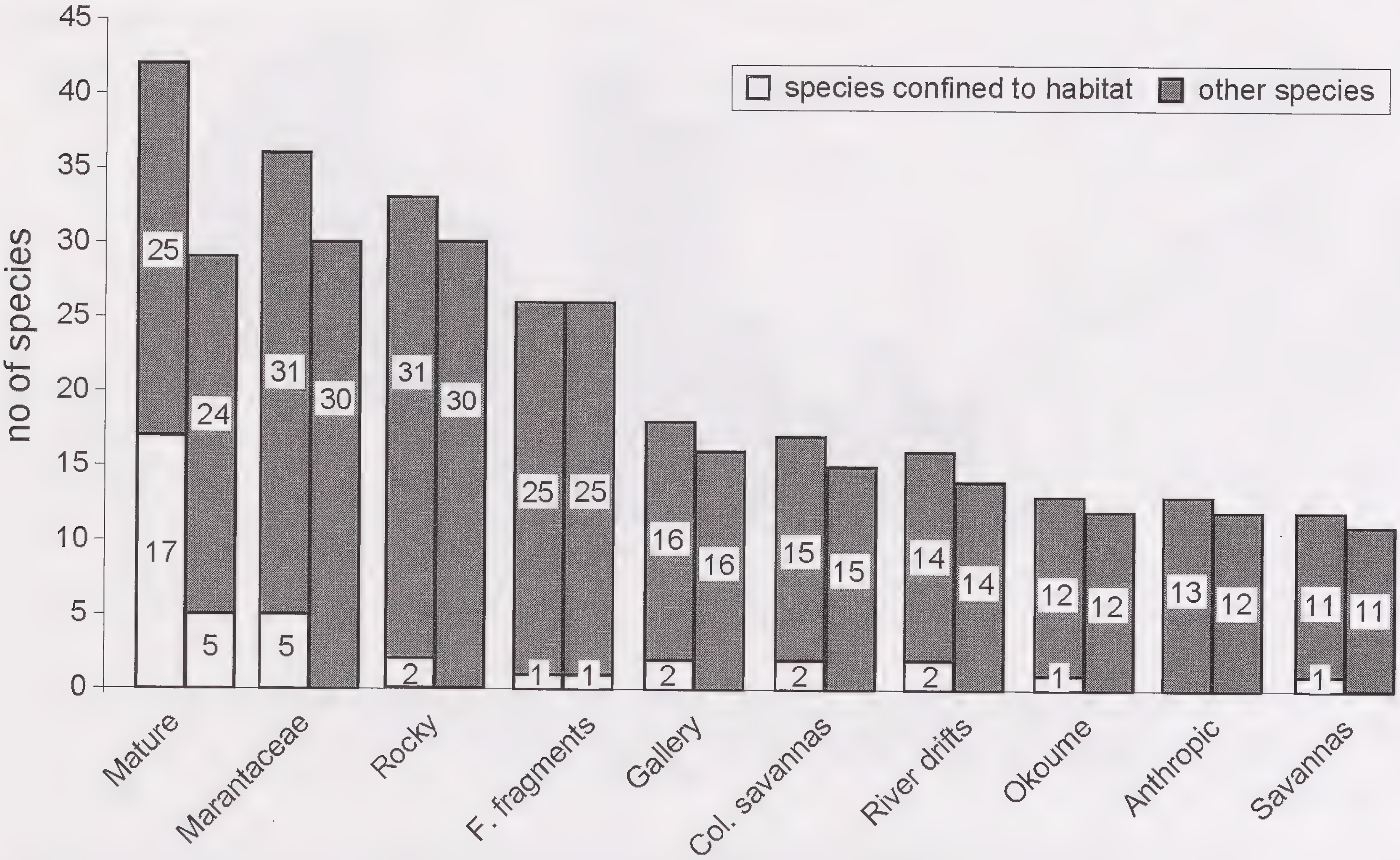

FIG. 9. Species diversity and ecological endemism in the various habitats of the Lopé National Park. For each vegetation type, the left bar include rare species (species present in more than two stations) whereas the right bar excludes them.

Four habitat types are much richer in species than the others (Fig. 9): mature forest, Marantaceae forest, rocky forest and forest fragments. Among these, mature forest has the highest species richness, as well as the highest number of species confined to a habitat: 57% of the fauna (42 species) occur in mature forest, and 23% of the total species are confined to this habitat. The other habitats have many fewer habitat-specific species. At the lower end, okoume forest, anthropogenic habitats and savannas are the least species-rich habitats. This is not linked to higher microhabitat diversity in mature forest: microhabitats as defined by us (between buttresses, leaf-litter in ground depressions etc.) were not more diversified in mature forest than in other forest types (Table 1). When rare species are excluded, this pattern does not change (Fig. 9). However, diversity in the various vegetation types is significantly correlated with sample size, measured by the volume of collected leaf-litter (r = 0.88, p = 0.0015): the more an habitat was sampled, the more species were collected.

There is some variation among the oldest forest types (i.e., mature and Marantaceae forest): we compared the fauna of these forests in the SEGC study area (43 stations) and further south, in Mikongo and/or along the Offoué River (21 stations). A high proportion of the SEGC fauna was not found in Mikongo/Offoué (Fig. 10). However, despite the fact that there were twice as many stations in the SEGC than in Mikongo/Offoué, 14.0% (eight species) of the species found in mature and Marantaceae forests were only found in Mikongo and/or along the Offoué River. Species found in both areas are more abundant on average than species found in one area only, and are

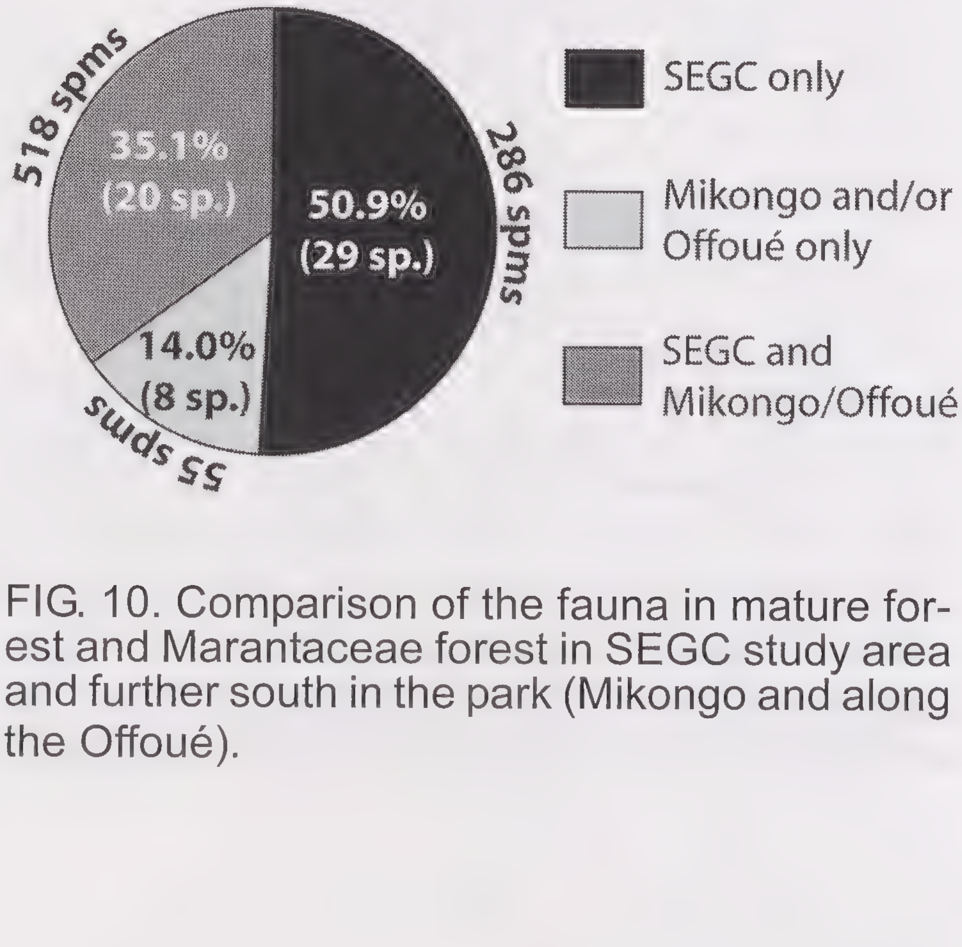

FIG. 10. Comparison of the fauna in mature forest and Marantaceae forest in SEGC study area and further south in the park (Mikongo and along the Offoué).

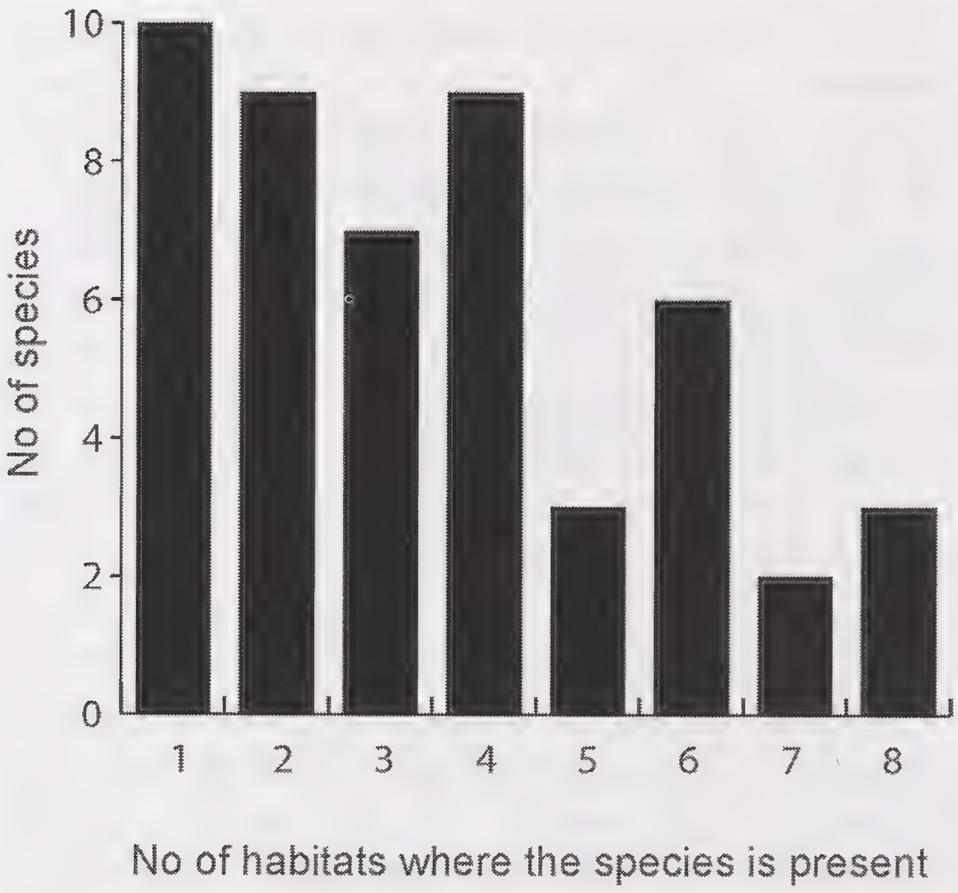

FIG. 11. Frequency distribution of the number of habitats for the 49 species found in more than one station. River drifts and anthropic habitats have been excluded.

thus easier to collect: that could account in part in the fact that they were found in both areas. However, species found in Mikongo/ Offoué only were not necessarily rare: four were found in more than one station, and three had 10 specimens or more. This implies that even in superficially similar habitats, the fauna can change on a 15 km scale. In conclusion, the high beta diversity is the result of both habitat mosaic and geographical location.

Three species were found in all eight habitats (excluding anthropogenic habitat and river drifts) and two others in all but one habitat (Fig. 11). Among the 49 species present at more than one station, 10 were found in only one habitat (one species in galleries, one in forest fragments, one in rocky forest, and the remaining seven in mature forest). A few species seem to be highly habitat-specialized, a few others are ubiquitous, and most species are intermediate, dwelling in a number of forest habitats. Twelve species were found in savannas, and only one of these was not found anywhere else. It is a biologically rare species (only one specimen found), and it might be present in other habitats despite our not finding it anywhere else. The other savanna-dwelling species were also found in various habitats, from mature forest to colonised savannas. Altogether, only two species were confined to savannas and "close to savanna" habitats (forest fragments and galleries).

No molluscs were found at six stations (two in mature forest, two in savanna, one in rocky forest and one in Marantaceae forest), and 13 stations produced only one species (five in mature forest, three in savanna, three in Marantaceae forest, and two in okoume forest). The two richest stations had 14 species each, and 12 stations had 10 or more species, in forest fragments, rocky forest, Marantaceae forest, mature forest, and anthropogenic habitat (respectively four, three, two, two and one station(s)).

The abundance of species (numbers of specimens) does not follow the same pattern as their diversity. Figure 12 shows that forest fragments constitute the richest habitat in term of abundance, even when the 602 specimens of *Subulona decollata* found on a single deviant station are discarded, followed by galleries and colonised savannas. Savannas and okoume forest are the least specimen-rich habitats, and the other forest types have an intermediate abundance of molluscs.

The six most abundant species in the study area belong to the Subulinidae (4 species), Urocyclidae (1 species), and Euconulidae (1 species). If station Gab159, where an extremely high number of *Subulona decollata* was found, is discarded, *Gudeella* sp. 2 (Urocyclidae) is the most abundant species.

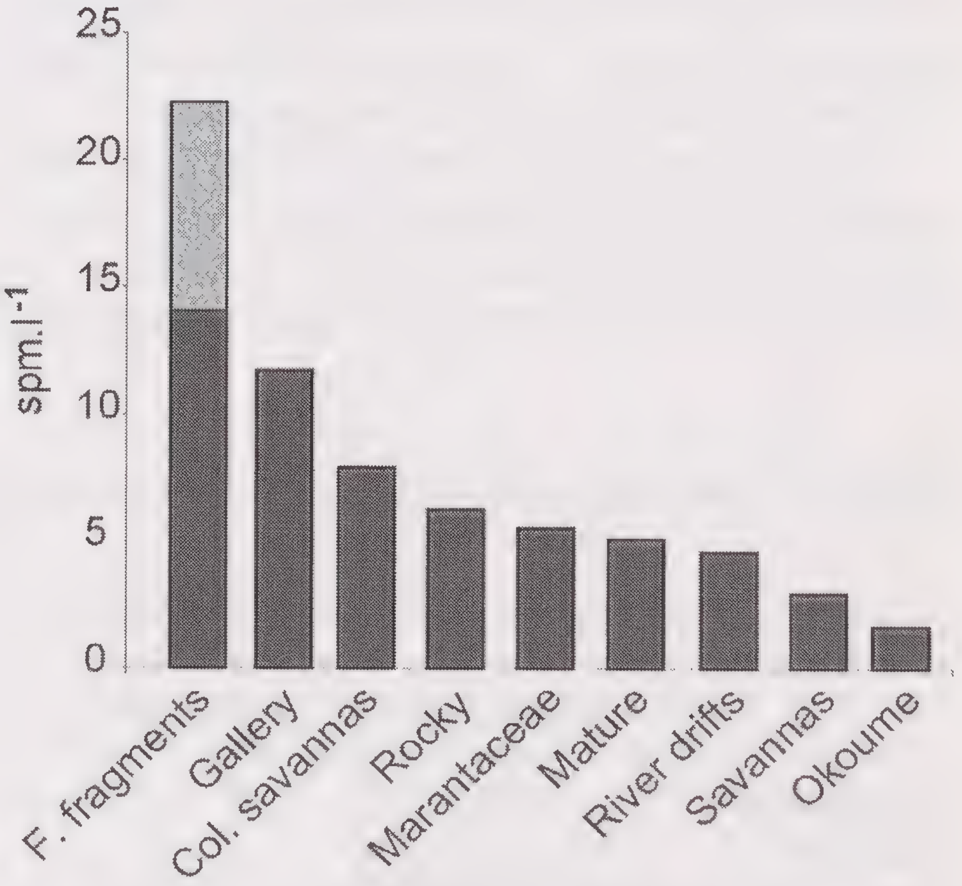

FIG. 12. Abundance of molluscs (number of specimens per volume of leaf-litter collected) in the various habitats of the Lopé National Park. The light grey area for forest fragments represents the 602 specimens of *Subulona decollata* found on station Gab159. Samples from anthropic habitat have been discarded since most of these were picked by hand, with no litter sieving. River drifts, although not representing a habitat, have been included for comparison.

Species Diversity in Microhabitats

The numbers of species found in each microhabitat are given in Table 3. Twenty-eight species (38%) were found in one microhabitat only, but 25 of these are rare species, found at only one station, and 36 species (48%) were found in three or more microhabitats. The species found at more than one station but always in the same microhabitat included two subulinids (*Ischnoglessula* sp. 1, three stations between buttresses, and *Striosubulina* sp. 4, three stations in leaf-litter) and a vertiginid (*Truncatellina* sp. 1, two stations between buttresses). Buttresses have the highest average number of species per station, though this is not significantly higher than for trees without buttresses (t-test, t = 1.47, df = 45, ns).

The rather unusual microhabitat of dry elephant bones yielded as many as 11 species (at one station): in this calcium-poor environment, bones probably represent an important source of calcium.

Seasonal Variation

Sampling took place during the rainy season and the long dry season. For the purpose of the seasonal variation analysis, sampling done in 1999 was discarded, because it occurred at the transition between dry and rainy season. The other sampling periods were well defined as occurring in one season only.

Sixty stations were sampled during the rainy season (267.2 L of litter collected) and 55 during the long dry season (119.8 L of litter collected). The total numbers of specimens were 2772 for the rainy season and 697 for the dry season, giving a mean number of specimens.L^{-1} of 10.4 for the rainy season and 5.8 for the dry season. Neither the number of specimens.L^{-1} nor the number of species.L^{-1} differs significantly between seasons (Mann-Whitney U test, ns).

The total number of species was higher in the rainy season (60 species) than in the dry season (49 species), although not statistically significantly, and slightly fewer than half of the species (46%) were found in only one season: 22 species were found only during the rainy season, and 11 only during the dry season. However, if we discard rare species (only one or two stations where the species was found), for which no conclusion regarding seasonality can be drawn, their being so scarce, three species were only found during the rainy season, and two only during the dry season. Among the 38 species that were found in both seasons, there are more species that are more common during the rainy season than during the dry season (22 vs. 16); 22 species (58%) had a relative abundance (standardized per litter volume) differing by more than 50% between seasons.

A separate analysis was performed for the twelve sites sampled twice: the median number of species per site, standardized per litter volume, is significantly higher at the beginning of the dry season than during the rainy season (Wilcoxon matched pairs test: z = 2.04, N = 13, p = 0.04). However, the median number of specimens per site (standardized per litter volume) is not significantly different between the seasons (Wilcoxon matched pairs test: z = 1.41, N = 13, ns).

TABLE 3. Number of species and stations per microhabitat.

Microhabitat	Number of species	Number of stations	Average number of species/station	Average number of specimens/station
buttresses	50	36	6.6 ± 3.9	42.5 ± 50.8
leaf-litter	47	50	4.3 ± 2.6	18.5 ± 17.8
base of trees without buttresses	34	12	4.8 ± 3.1	23.8 ± 20.9
rotten logs	27	17	3.7 ± 2.6	9.8 ± 15.25
rock crevices	22	7	3.7 ± 3.1	14.0 ± 20.9
elephant bones	11	1		
house walls	6	4	2.3 ± 1.5	2.3 ± 2.3
tree trunks	1	1		
bare ground	1	4		

Faunal Assemblages

In order to estimate if snail faunal assemblages match vegetation types (White et al., 1995), we performed a Principal Components Analysis with a matrix of species within stations. However, no obvious pattern was evident, the abundance and ecological rarity of species being the main factors generating the clustering, even when rare species were removed.

A cluster analysis of a presence/absence matrix of species within stations did not produce any obvious pattern either. The main factor determining clustering was the number of species within stations. Among the species-poor stations, all the mature forest stations above 400 m asl were clustered together, with other mature forest stations from lower areas. Geographical proximity was not a factor determining clustering (stations from Mikongo are spread all over the dendrogram), neither was the type of microhabitat. However, the clustering distances was low, and the faunal partitioning did not seem to be determined by the vegetation types seen by botanists.

DISCUSSION

Habitat Diversity

No work has been published in a scientific journal on the plant richness in the various forest types in the Lopé National Park. White (1992) had some data, but these are now completely outdated because new inventories have been made (White, pers. comm.). However, White et al. (1995) quote the fact that older forest types are richer than younger ones, and scientists working in Lopé confirm this fact (White, pers. comm.; Tutin, pers. comm.). Not surprisingly, mollusc species richness seems to follow plant species richness (Fig. 9), the richest habitats for snails being the most bo-

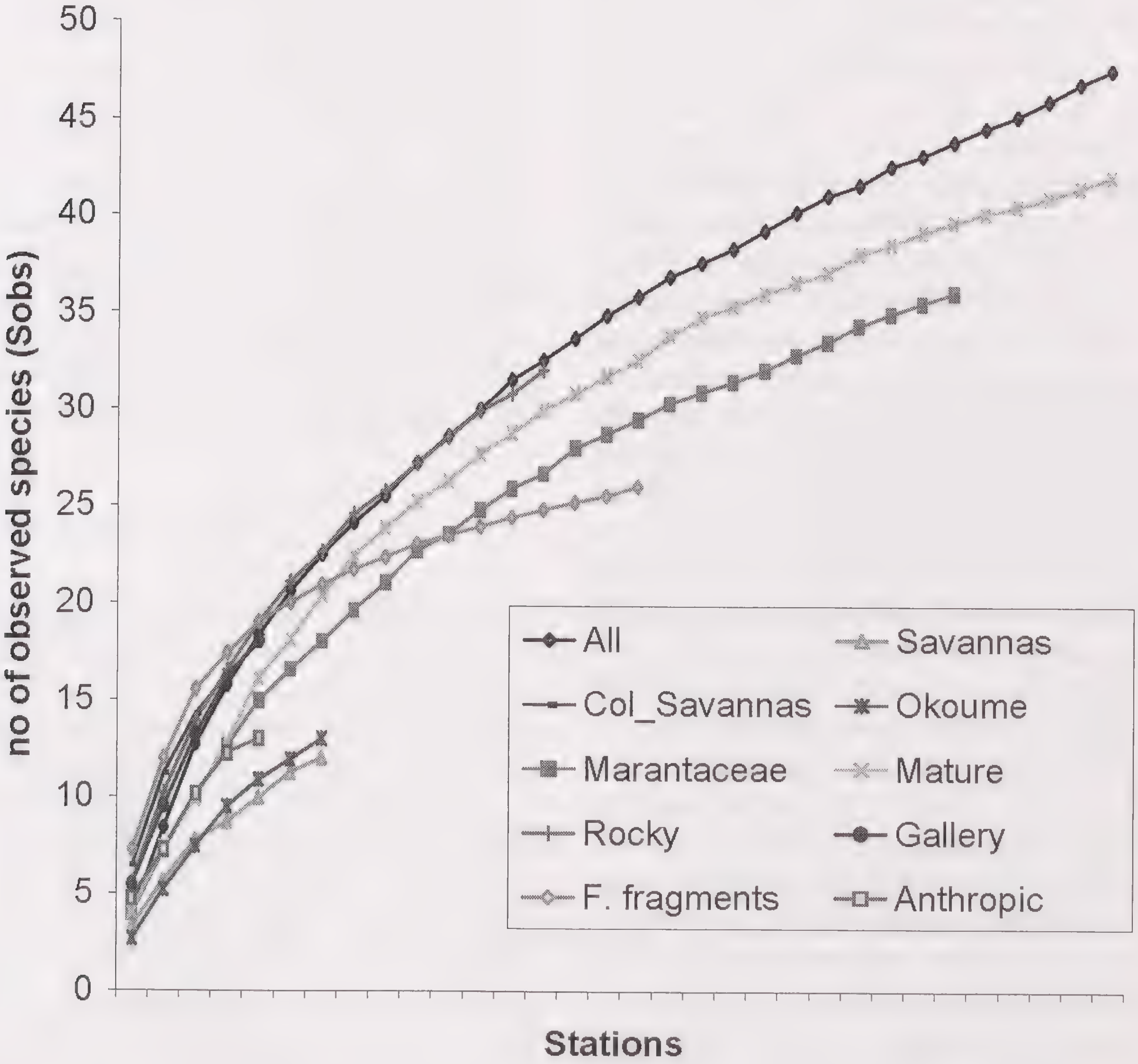

FIG. 13. Species accumulation curves for various habitats based on EstimateS 7.5 (Colwell, 2005). River drift sites have been discarded, not representing a true habitat.

tanically diverse vegetation types, that is, mature and Marantaceae forest, which are at the end of the succession from savannas to old forests, and rocky forest and forest fragments, which are out of this succession but are old vegetation types. Rocky forest is a very rich habitat for molluscs, as shown by Simpson's index (Table 2); even with a lower collecting intensity than in forest fragments, it is more diverse. As we defined microhabitats, our analyses do not show an association of species and microhabitats, and as they are similarly distributed in the various forest types, they cannot account alone for the variation in our data. If microhabitats had been more finely defined (e.g., pH, substratum, humidity, exposure), it might have been possible to examine whether they can explain species distribution. This was not the case in our sampling protocol, so it is safer to discuss species diversity and associations at the habitat level, be it determined by microhabitats, precise floral composition or other factors.

However, the ranking of vegetation types is biased by the fact that the various vegetation types were not sampled with the same intensity (Table 1). For example, galleries and colonised savannas seem to be poorer than other vegetation types, but they also had a lower collecting effort (fewer stations were prospected there than in mature or Marantaceae forests). Indeed, diversity in the various vegetation type is significantly correlated with sample size (measured by number of stations or volume of collected leaf-litter). To account for this bias, a species accumulation curve was constructed for each habitat (Fig. 13). As most of these curves do not show any evidence of flattening, a measure of the confidence of the richness estimates is given Figure 14. Mature forest has the highest number of species, followed by Marantaceae forest, rocky forest, and forest fragments. However, for rocky forest, a larger number of sampled stations would have permitted confirmation of this tendency. Okoume forest and savannas are the poorest habitats, falling well below the other curves. This is not surprising: savanna is a harsh habitat, dry and sunny, with almost no organic soil and few sheltering microhabitats; okoume forest is much more homogeneous than the other forest types, and the low diversity of other organisms, including plants, might well be reflected in the low diversity of molluscs. Nevertheless, these two habitats, as well as galleries, colonised savannas and anthropogenic habitats were not sampled enough to draw definite conclusions from these curves. From these results, it is however clear that the savannas in Lopé do not have a specific fauna: except for one rare species (*Pseudopeas* sp. 3), no species was restricted to savannas. The savannas in Lopé are isolated in a sea of forest, the closest savannas being 200 km away, and are subject to frequent fires (usually every second or third year). Most species in savannas also live in other forest types, and probably recolonize the savannas from the forest after burning episodes. On a larger scale (the whole of southern Africa), Bruggen (1978) drew similar conclusions, that is, southern African savanna-dwelling land molluscs are de-

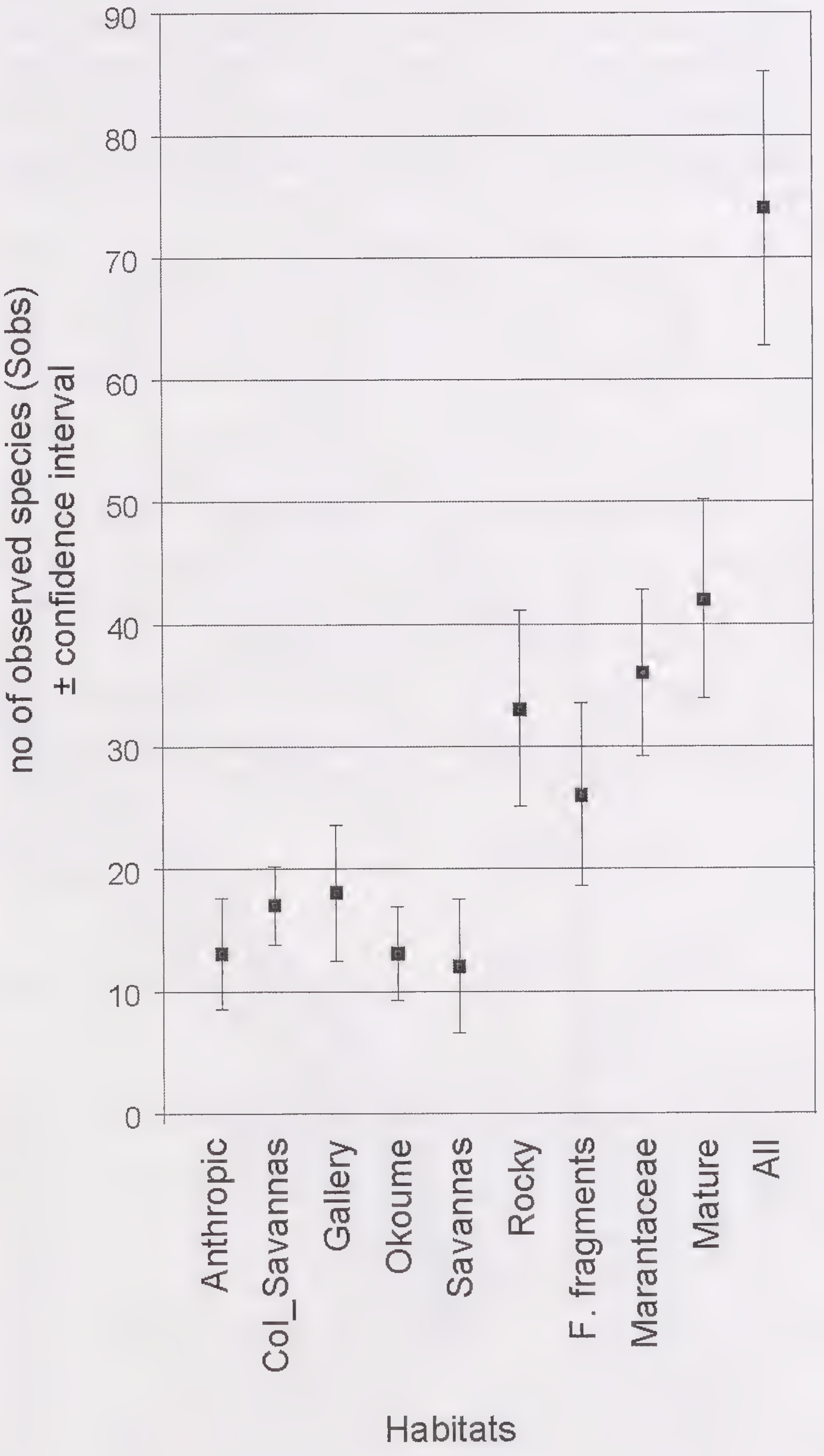

FIG. 14. Number of species expected in the pooled samples for various habitats with 95% confidence intervals, based on EstimateS 7.5 (Colwell, 2005). Habitats are ranked according to the increasing number of stations.

rived from, or are ecologically widely tolerant, forest taxa.

There is also some variability within habitats, as shown by the comparison between the mature and Marantaceae forests of the SEGC study area and the Mikongo/Offoué area. Simpson's index for mature forest in Lopé is 18.66, which is high, but might be influenced by the sampling not being exhaustive, with many biologically or ecologically rare species.

Striosubulina sp. 7 is among the most abundant species in forest fragments, galleries and savannas, and, except for one station in Marantaceae forest, less than 100 m from the forest edge, has not been found in other habitats (particularly forest). This species is commonly found in plantations, gardens and urban areas all over Gabon (BF, pers. obs.): it is probable that in Lopé, it has followed the human population in ancient settlements (destroyed a long time ago an now turned into forest fragments) and has spread in the open habitats around them. On the other hand, *Gudeella* sp. 2, which is also one of the most abundant species in forest fragments, galleries, colonised savannas and savannas, has also been found at several stations in Marantaceae forest, mature forest, and rocky forest (three, seven and four stations respectively): it is a generalist, living in all the habitats from open savanna to dense mature forest. However, the possibility that *Gudeella* sp. 2 is in fact a complex of closely related species should not be excluded, its shell having few characteristic features and no anatomical studies having been performed.

Seasonal Variation

Our data show no significant difference in either abundance or diversity of land snails between the rainy and dry seasons. Indeed, the so-called "dry" season has the same level of atmospheric humidity as the rainy season (Tutin & Fernandez, 1993). There is dew in the morning all year long, and land snails probably do not suffer from a shortage of water in the "dry" season: for the period 1984–2002, on average, 10.4% (154.8 mm) of the annual rainfall occurred during the long dry season (SEGC, unpublished data). However, some species seem to be more abundant during one part of the year, probably because of such behaviour as aestivation/hibernation (animals are more concealed part of the year), or because they have a life cycle of one year or less. The fact that in the twice-sampled sites, the number of species is significantly higher at the beginning of the dry season than during the rainy season also suggests that there could be seasonality in the life cycle of some species. However, we cannot rule out the fact that results from the sites sampled twice could be influenced by disturbance, or recolonisation factors following the first sampling. The paucity of data regarding life history of land snails in tropical Africa prevents any firm conclusions on seasonality, but similar results have been found in other studies (De Winter & Gittenberger, 1998).

The results regarding seasonality are biased by the fact that sieving the leaf-litter produces many dead individuals (empty shells), which are not necessarily representative of the fauna at a given time of the year. Seasonality would be better studied with live individuals only, but as was indicated in the Methods section, it is difficult and time-consuming to sort out animals collected alive from those collected dead, especially for minute species.

Shell Size and Shape

The distribution of H/D is very similar to that presented by De Winter & Gittenberger (1998), but very different from that for the faunas of sites in Madagascar, New Zealand, and USA (Emberton, 1995), which are all unimodal with a peak in the 0.4–0.8 class (flat shells). Our fauna includes a large proportion of globose shells, mainly Urocyclids (*Teleozonites* spp. and *Trochozonites* spp.). However, more than 70% of our species are tall (H/D > 1.0), reflecting the abundance of Streptaxidae and particularly Subulinidae. This abundance of tall species contrasts with the faunas presented by Emberton (1995). Cain (1977) suggested that on average, high-spired species would favour vertical surfaces (trees, rocks), whereas low-spired species would tend to live on the ground. In Lopé, where we did not find truly arboreal species, a high proportion of the ground-foraging species are high-spired, contrary to Cain's hypothesis.

The range of shell size in Lopé is huge, from 1.3 mm to 118.6 mm, but most species are small to minute. The proportion of minute species (*sensu* Emberton, 1995), shell diameter 0–5mm) is even higer than in the Cameroon site (De Winter & Gittenberger, 1998) or the Madagascar site (Emberton, 1995) (74% vs. 66% and 63% respectively), but the overall pattern of size distribution is the same, with very few large species. Together with the rarity of many species, this emphasizes the fact that mollusc sampling cannot be done with the naked eye only, and should be done with litter sieving, the fastest way to collect minute species in significant numbers.

Streptaxids as Predators

As reviewed by De Winter & Gittenberger (1998), carnivorous streptaxids are a prominent part of the malacofauna of the Afrotropical region, representing between 18% (Tattersfield, 1996) and 46% (Emberton et al., 1997) of the fauna. Lopé is no exception to this situation, with streptaxids representing 26% of the species found, but as much as 38% of the biologically rare species and 34% of the ecologically rare species. On the other hand, subulinids, which represent 35% of the species, constitute only 20% of the biologically rare species and 26% of the ecologically rare species; urocyclids, which represent 16% of the species, constitute 17% of the biologically rare species and 18% of the ecologically rare species. When the number of specimens is considered, streptaxids constitute 8% of the total number of specimens, subulinids 63% and urocyclids 14%. The relative rarity of streptaxids compared to subulinids and urocyclids might be linked to their feeding ecology, streptaxids being carnivorous whereas subulinids and urocyclids are phytophagous, but data supporting this speculation are lacking. However, a single streptaxid species – *Streptostele musaecola* (Morelet, 1860) – is both abundant (126 specimens found) and widely distributed, in various habitats (28 stations, seven habitats). *Streptostele musaecola* was described from Africa (Morelet, 1860), but is known to be an invading species, with a circumtropical distribution (Solem, 1988b; Hausdorf & Medina Bermudez, 2003), and is probably highly adaptable.

Richness and Diversity

In its overall composition, the malacofauna of Lopé resembles that of rainforest in southwestern Cameroon (De Winter & Gittenberger, 1998) and in another part of Gabon (De Winter, 1995), the most speciose families being Subulinidae, Streptaxidae, and Urocyclidae. However, streptaxids are the most speciose family in Cameroon, whereas subulinids are the most speciose family in Lopé.

The Lopé malacofauna comprises at least 74 species in approximately 50 km², and up to 132 species according to the Chao2 richness estimator (Fig. 4). These figures are in the same order of magnitude as results of other studies in tropical Africa. The richest site known to date in a tropical environment is in Cameroon, with 97 species (and up to 108 according to the richness estimators) in 1 km² of rainforest (De Winter & Gittenberger, 1998). This high diversity is further emphasised by the fact that "only" 2,654 specimens were collected at the Cameroon site, compared to 3,745 in our study, and that there was only one type of macro-habitat in the Cameroon site, that is, very old secondary forest. In 265 km² of indigenous forest and secondary vegetation in Kenya, Tattersfield (1996) found 53 species, and estimated that there should be 70–80 species in total. In the rainforest of two mountain ranges of southeastern Madagascar, Emberton et al. (1999) reported 80 species, though they did not pretend to have made a complete inventory. The only other study in Gabon (De Winter, 1995) listed 32 species in 48 km² of rainforest, but they were collected by a "malacologically inexperienced botanist" during floristic transects, so this inventory is probably far from complete. In tropical America, Gargominy & Ripken (1998) found 32 species in 1 km² of rainforest. On a smaller scale, Rosenberg & Muratov (1998) found 73 species of terrestrial molluscs in 4 ha in Jamaica; this diversity is probably linked to the site being in a karst area, where molluscan diversity and abundance are always high. In Cameroon, De Winter & Gittenberger (1998) found 83 species on 9 ha of rainforest, a huge diversity for acidic soils.

At the Cameroon site, 27 species (28% of all species) were found only on understorey vegetation, at 75 cm or more above the ground. In a study using the same methodology (beating of understorey vegetation) in Sabah (Schilthuizen & Rutjes, 2001), 11% (7 species) of the fauna was strictly arboreal. Our collecting method did not involve systematic beating of understorey vegetation, but we never found snails on the leaves of understorey plants, and very rarely on tree trunks. Whether this lack of arboreal fauna represents a sampling bias or is real, it accounts at least in part for the lower richness of Lopé compared to Cameroon.

Another reason for this lower richness could lie in the vegetation history. The area we studied, in the northern part of the Lopé National Park at the edge of the savanna, is mostly composed of "young" forest, resulting from the extensive recolonisation of savannas that occurred between 1500 and 700 years BP, when humans left the area and fires were rare (Oslisly & White, 1996; White, 2000). Unlike the Cameroon example, the forest of the northern part of the Lopé National Park is not a Pleistocene forest refuge (Maley, 1996), which might be related to the lower number of species found in Lopé compared to the Cameroon site. This could also account for the fact that arboreal snails are less abundant in Lopé than in the Cameroon site, as these are the most likely to be dependent on long periods of uninterrupted humid forest cover, and would have disappeared when the forest was replaced by savannas. On the other hand, the diversity of habitats and the presence of ecotones probably account for the fact that Lopé still ranks among the richest sites. However, outside the tropics, restricted areas in Australia, New Zealand, and the Carpathians mountains can also have a fauna of more than 70 species (R. Cameron, comm. pers.).

Sampling Problems

The value of our Whittaker's index is very high compared to other similar studies (compilated in Cameron & Pokryszko, 2005). However, the average number of specimens per station is lower than the total number of species collected during this survey: it is not possible for all species to be recorded at each station. Thus, sampling errors (i.e., the failure to find a species in a station) most probably account for this high value, together with habitat heterogeneity. Moreover, species richness in various habitats is significantly correlated with the amount of collected leaf-litter (i.e., with sampling intensity), as is the case in other studies (Cameron & Pokryszko, 2005). This is a further indication that sampling was not exhaustive, because if all species had been collected, more sampling would not add more species. Implications of sampling differences between habitats have been discussed above, but they are difficult to avoid if the aim is to perform an exhaustive inventory: in the field, one checks in priority habitats that should yield the best results, and poorer habitats are given less attention.

Towards an Ideal Strategy for a Maximum Efficiency of Tropical Forest Molluscan Biodiversity Inventories

All the studies cited above use a combination of direct search and litter sieving (except De Winter, 1995), which allows the most efficient inventorying, as was stressed by several authors (Emberton et al., 1996; Cameron & Pokryszko, 2005). The amount of time spent searching on the spot varied according to the available manpower, usually between one and three person-hours. Not everybody can afford 66 people collecting during a whole day, totaling 450+ person-hours (Emberton, 1995)! The volume of litter collected varied between 4 L and 8 L per station in the various studies.

Molluscs are not uniformly distributed in the forest: our sampling shows that some areas are devoid of molluscs, whereas others, in the same macrohabitat, exhibit high richness and abundance. The importance of the microhabitat cannot be underestimated, and can lead to sampling biases. Random sampling would have led to a much lower number of specimens being found, as is suggested by (1) the paucity of specimens from our (few) randomly located sampling sites, and (2) the variability of the number of species found in three stations less than 15 m away from each other (between the buttresses of a *Ceiba pentandra*, 12 species; between the buttresses of an okoume, nine species; in a depression on the floor, four species). Other studies have shown that random quadrats alone are not an efficient method for inventory (Cameron & Pokryszko, 2005). On a meso scale, our sampling was random (choice of the general area in a given habitat), but the microhabitats (between buttresses, under logs, in depressions) were carefully chosen.

The importance of carefully choosing the sampling spots is emphasized by the results of the other study in Gabon (De Winter, 1995), where the choice of the stations was random (following floristic transects), and not aided by a malacologically experienced eye. The diversity of the fauna found during this study was low, compared to our own or the Cameroon one, given that the habitat is a central African undisturbed lowland rain forest in all three studies. It seems certain that careful collecting done by an experienced collector would add many species.

In a given area, the choice of the habitat sampled is also important. Old undisturbed vegetation types should be given priority, for they usually harbour a higher diversity in tropical environments: the richest sites known to date are in these types of habitats (this study; De Winter & Gittenberger, 1998; Emberton et al., 1999; Schilthuizen & Rutjes, 2001). Other habitats should also be sampled, and may yield species that are restricted to them. But if the aim is to maximise the inventory, rather than to perform an ecological comparison, old undisturbed vegetation types should be sampled with a higher intensity than other habitats.

The inventory of a given area should not be extrapolated to the surrounding region. As shown by the differences of fauna between Mikongo and the SEGC area in our study, apparently homogeneous habitat can harbour different faunas at a 15 km scale. This result is comparable to that found in Cameroon: in three similar sites (30 km apart at the most), 32% of the malacofauna was present in only one of the sites (De Winter, 2001). Similar results have been found in coral reefs (Bouchet et al., 2002). In this respect, to achieve the most exhaustive inventory for an area, sampling should not only cover the various habitats, but also be spread over the entire area to account for geographical variation.

Last but not least, considering the generally high proportion of ecologically rare and small species, it is necessary to collect a lot of litter. For Europe, 20 l of leaf litter are considered adequate to sample the fauna of oligotrophic sites (Cameron & Pokryszko, 2005), it is probably a minimum for sites such as Lopé. The sorting is time-consuming, but flotation is an efficient way of reducing the amount of material to be sorted.

Limits of the RTU Approach

The main purpose of the RTU (morphospecies) approach is to circumvent the taxonomic impediment. In most cases, a classical approach, with full taxonomic identifications, could never be done in a reasonable amount of time, because of the lack of expertise and funding. This new way of dealing with tropical faunas and the use of proper collecting techniques are responsible for the complete shift of opinions regarding molluscan biodiversity in tropical forests. The studies cited above, which have changed our vision compared to that of Solem (1984), that is, generally low diversity of land snails in rain forests, were published with a high proportion of species unidentified.

The use of RTUs in biodiversity studies has been criticized (Slotow & Hamer, 2000; Krell, 2004), mainly because morphospecies sorting is usually done by “parataxonomists”, who do not have good knowledge of the specific taxonomic characters of the study group. Their results are not always reliable, often lead to overestimation of the number of species and the accuracy can be very low. However, our approach has avoided those problems because the sorting has been done by an experienced mollusc taxonomist (E.N.), who has followed the usual steps of taxonomic processing of samples, but stopped the process before giving names to RTUs. The samples have been placed in an accessible collection, namely that of the Muséum National d’Histoire Naturelle, Paris.

However, the RTU approach has some drawbacks:

- The RTUs have significance only for the people who have created them: a list of RTUs is useless to anyone else (only the number of RTUs can be used by others). This approach does not allow comparisons of the composition of faunas studied by different authors. The range of an RTU outside the study area cannot be known, and in particular, it is not possible to work on endemism, as the literature cannot be used to know whether a given RTU occurs somewhere else. This point is important from a conservation perspective. As was highlighted by Slotow & Hamer (2000), the number of RTUs in a given area is of little help for conservation planning, as these can be widespread and common species.
- If the study highlights some concerns about the conservation of a given RTU, it is impossible to take legal measures at the species level: to be listed in a Red List or a protected species list, a species has to have a recognised name. In the case of invertebrates, it is often more efficient to work at the habitat level than at the taxon level; however, the importance of Red Lists or protected species lists should not be underestimated. Names are a key to get access to scarce resources such as funds and expertise.
- When RTUs are used, it is impossible to know what proportion of a fauna remains to be

described. In some groups, this proportion can be huge (up to 80%, for instance in Bouchet et al., 2002).

In order to be able to validate the significance of RTUs, voucher material must be placed in an appropriate accessible collection (New, 1999).

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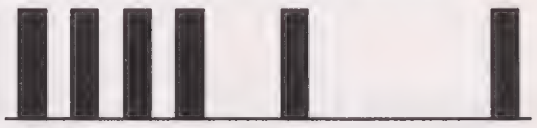
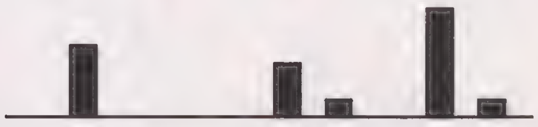
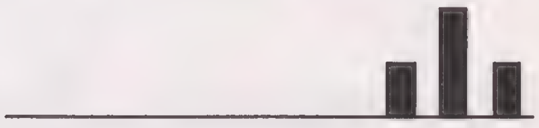
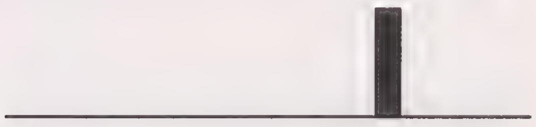
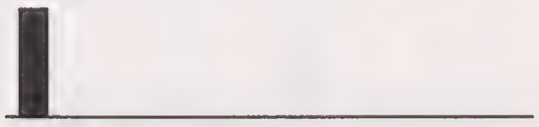
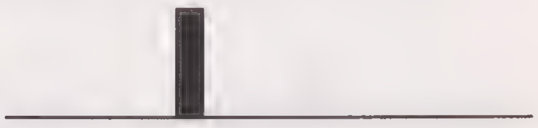
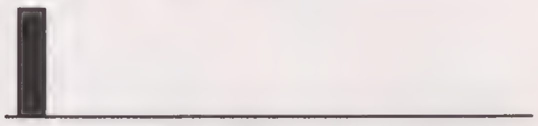
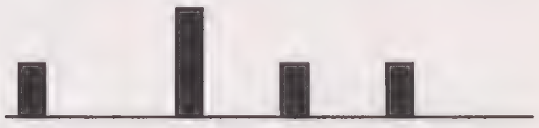
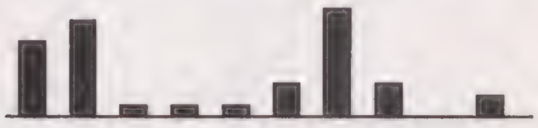
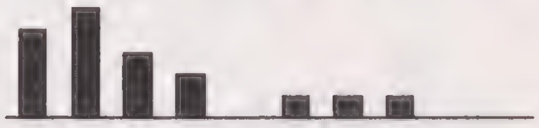
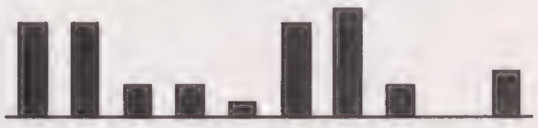
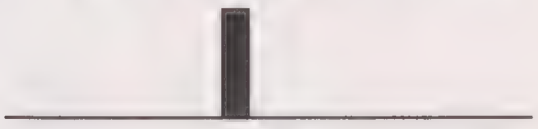
APPENDIX

List of land snail taxa recorded in the present study with total numbers of collected specimens, total number of stations where the taxon was found, shell morphometrics (height, diameter, and height-diameter ratio, all measured from randomly selected adult specimens). Right-hand column: bars indicate for each species the proportion of stations in each habitat type.

Taxon	Family	Total specimens	Total stations	Height (mm)	Diameter (mm)	H/D	MATURE MARANTACEAE OKOUME COL_SAVANNAS SAVANNAS ROCKY FOREST_FRAGM RIVER_DRIFTS ANTHROPIC GALLERY
Maizaniella (Spiruloziana) lilliputiana (Morelet, 1873)	Maizaniidae	67	6	2.2	3.4	0.65	
Veronicellidae spp.	Veronicellidae	17	12	-	-	-	
Quickia sp. 1	Succineidae	26	3	5.4	3.8	1.42	
Pupisoma sp. 1	Valloniidae	2	1	1.7	1.5	1.13	
Truncatellina sp. 1	Vertiginidae	6	2	1.6	0.8	2.00	
Nesopupa sp. 1	Nesopupidae	1	1	1.3	0.7	1.86	
Nesopupa sp. 2	Nesopupidae	1	1	1.4	0.8	1.75	
Subulinidae ? sp. 1	Subulinidae	10	5	3.7	1.2	3.08	
Pseudopeas sp. 1	Subulinidae	208	37	5.9	2.4	2.46	
Pseudopeas sp. 1a	Subulinidae	75	17	5.4	2.5	2.16	
Pseudopeas sp. 2	Subulinidae	120	35	4.2	1.7	2.47	
Pseudopeas sp. 3	Subulinidae	1	1	3.1	1.3	2.38	

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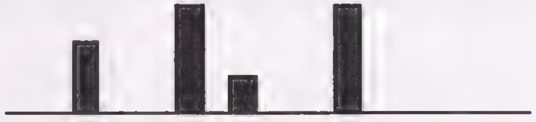
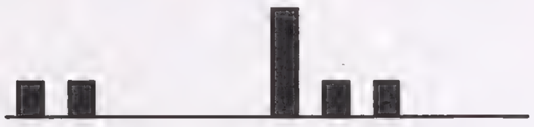
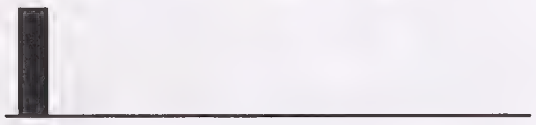
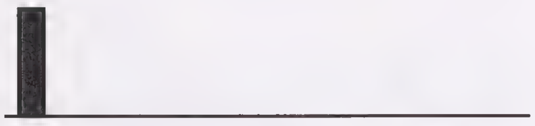
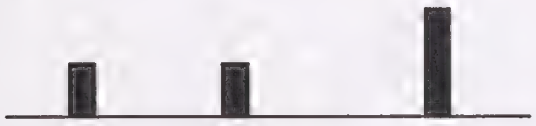
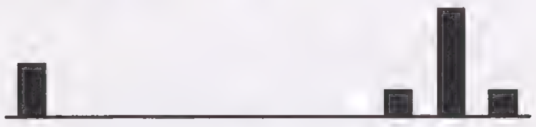
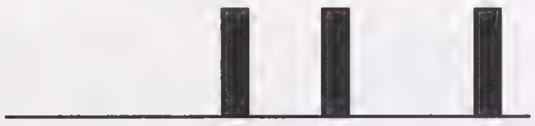
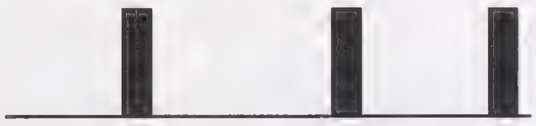
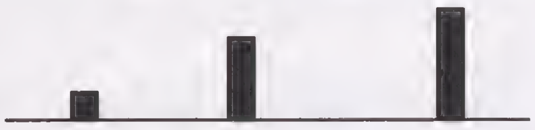
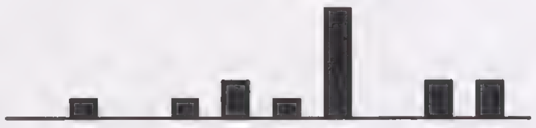
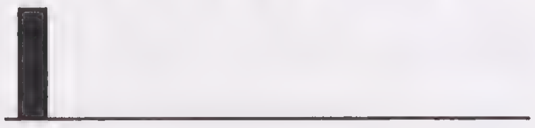
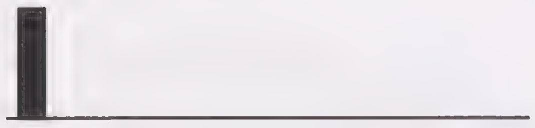
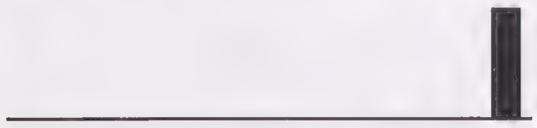
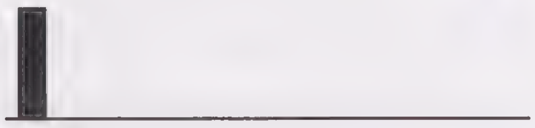
Taxon	Family	Total specimens	Total stations	Height (mm)	Diameter (mm)	H/D	MATURE MARANTACEAE OKOUME COL_SAVANNAS SAVANNAS ROCKY FOREST_FRAGM RIVER_DRIFTS ANTHROPIC GALLERY
Pseudopeas sp. 4	Subulinidae	41	9	4.1	2.2	1.86	
Pseudopeas ? sp. 5	Subulinidae	22	7	3.2	1.2	2.67	
Curvella sp. 1	Subulinidae	10	4	5.1	2.4	2.12	
Curvella sp. 2	Subulinidae	11	6	5.4	2.2	2.45	
Striosubulina sp. 1	Subulinidae	6	3	12.2	3.8	3.21	
Striosubulina sp. 2	Subulinidae	142	6	15.4	3.7	4.16	
Striosubulina sp. 3	Subulinidae	4	3	17.2	4.8	3.58	
Striosubulina sp. 4	Subulinidae	23	3	18.6	5.8	3.21	
Striosubulina sp. 5	Subulinidae	52	6	15.1	4.1	3.68	
Striosubulina sp. 6	Subulinidae	221	14	16.4	4.33	3.73	
Nothapalus? sp.	Subulinidae	1	1	14.8	5.4	2.74	
Dictyoglessula sp. 1	Subulinidae	6	3	8.2	4.3	1.91	
Oleata? sp. 1	Subulinidae	2	1	15	4.7	3.19	
Oleata? sp. 3	Subulinidae	4	1	10.3	4.6	2.24	

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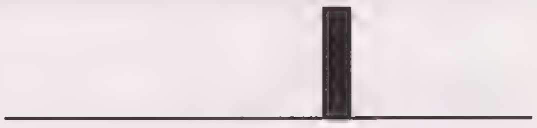
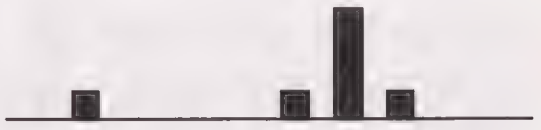
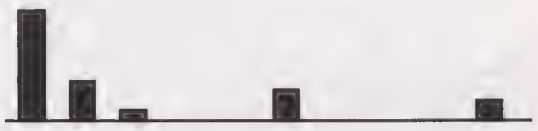
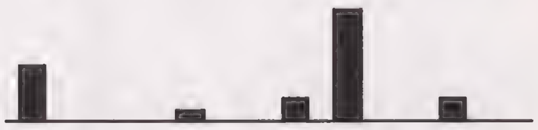
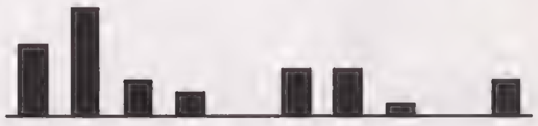
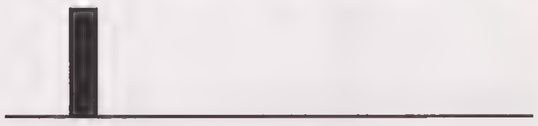
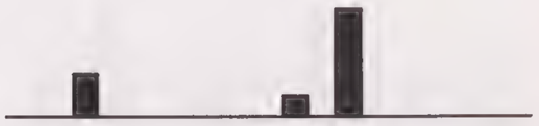
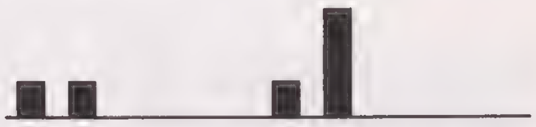
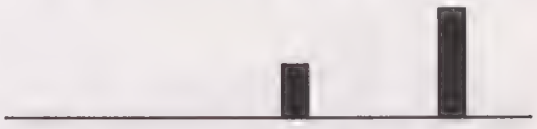
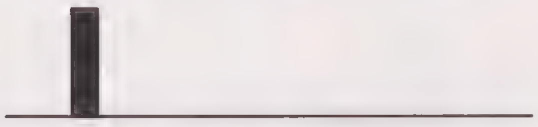
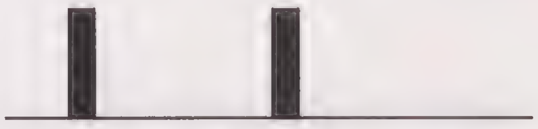
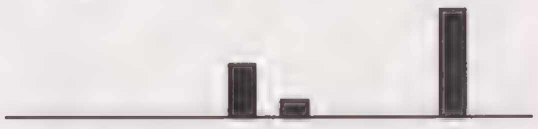
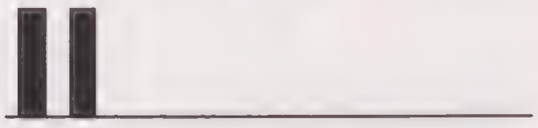
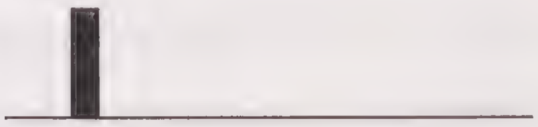
Taxon	Family	Total specimens	Total stations	Height (mm)	Diameter (mm)	H/D	MATURE MARANTACEAE OKOUME COL_SAVANNAS SAVANNAS ROCKY FOREST_FRAGM RIVER_DRIFTS ANTHROPIC GALLERY
Ischnoglessula sp. 1	Subulinidae	32	3	8.8	2.8	3.14	
Ischnoglessula sp. 3	Subulinidae	22	7	10	2.9	3.45	
Subulona sp. 1	Subulinidae	139	21	10.8	2.4	4.50	
Subulona decollata (Morelet, 1873)	Subulinidae	759	19	23.2	6.6	3.52	
Pileata sp. 1	Subulinidae	136	32	17.6	6	2.93	
Pileata sp. 2	Subulinidae	2	1	20.4	5.5	3.71	
Kempioconcha sp. 1	Subulinidae	220	8	9.8	4.8	2.04	
Cecilioides sp. 1	Ferussaciidae	11	6	1.9	0.7	2.71	
Achatinidae gen. sp. 1	Achatinidae	2	2	36.1	18.3	1.97	
Achatina balteata Reeve, 1849	Achatinidae	1	1	118.6	62.2	1.91	
Leptocala mollicella	Achatinidae	5	4	24.7	14.7	1.68	
Limicolaria sp. 2	Achatinidae	24	7	41.1	18.4	2.23	
Streptaxidae sp. 1	Streptaxidae	6	2	2	3.5	0.57	
Edentulina sp.	Streptaxidae	1	1	5.9	4.6	1.28	

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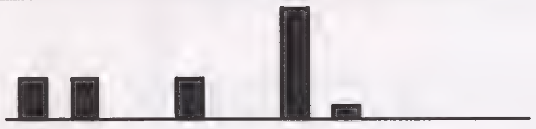
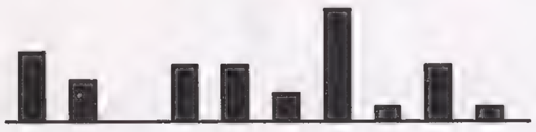
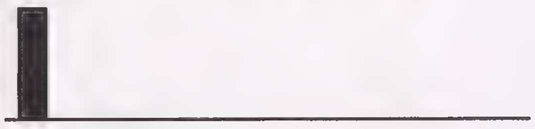
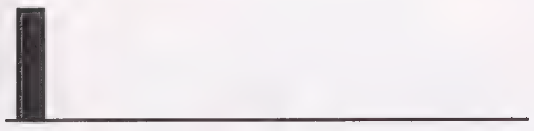
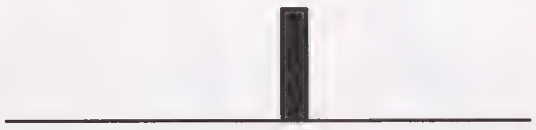
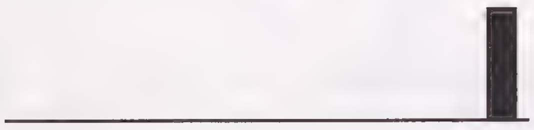
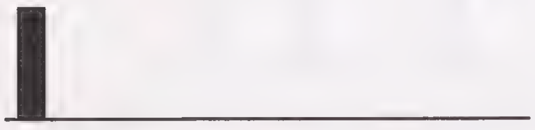
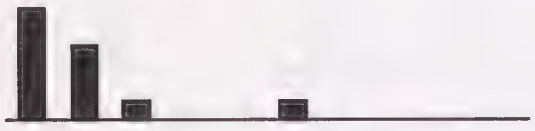
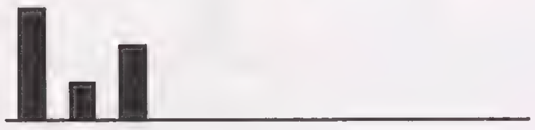
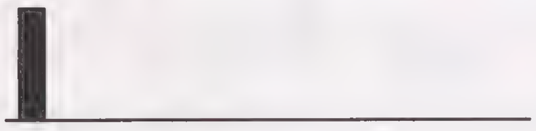
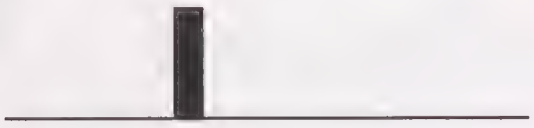
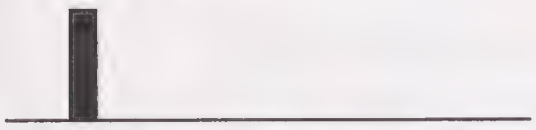
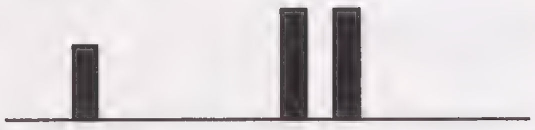
Taxon	Family	Total specimens	Total stations	Height (mm)	Diameter (mm)	H/D	MATURE MARANTACEAE OKOUME COL_SAVANNAS SAVANNAS ROCKY FOREST_FRAGM RIVER_DRIFTS ANTHROPIC GALLERY
Streptostele sp. 3	Streptaxidae	79	18	13.1	3.6	3.64	
Streptostele (Tomostele) musaecola (Morelet, 1860)	Streptaxidae	124	30	5.9	1.7	3.47	
Varicostele sp. 3	Streptaxidae	10	4	6.2	2.6	2.38	
Gulella s. lat. sp. 2	Streptaxidae	1	1	8.5	4.4	1.93	
Gulella (Avakubia) sp. 1	Streptaxidae	1	1	4.5	1.9	2.37	
Gulella (Avakubia) sp. 2	Streptaxidae	2	1	3.1	1.6	1.94	
Gulella (Paucidentina) sp. 1	Streptaxidae	11	2	2.9	1.5	1.93	
Gulella (Paucidentina) sp. 2	Streptaxidae	22	12	4	1.7	2.35	
Gulella (Paucidentina) cf. *monodon* (Morelet, 1873)	Streptaxidae	9	6	8.4	3.9	2.15	
Gulella (Paucidentina) sp. 4	Streptaxidae	1	1	3.0	2.9	1.03	
Parennea (Parennea) sp. 4	Streptaxidae	1	1	2.7	1.3	2.08	
Ptychotrema (Ennea) sp. 3	Streptaxidae	1	1	5.1	2.3	2.22	
Ptychotrema (Ennea) sp. 4	Streptaxidae	23	8	3.5	1.8	1.94	

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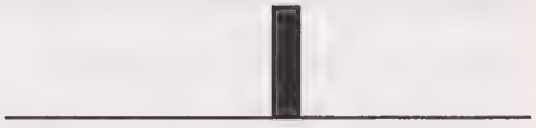
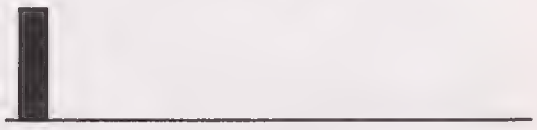
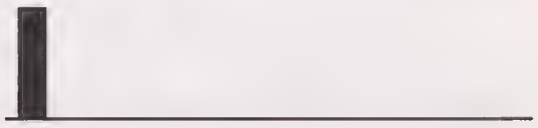
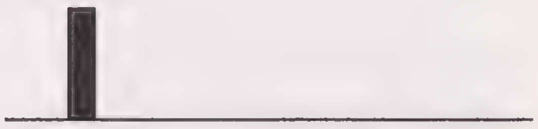
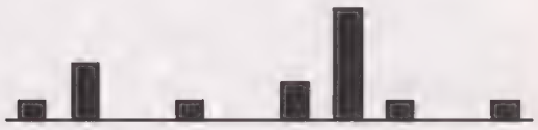
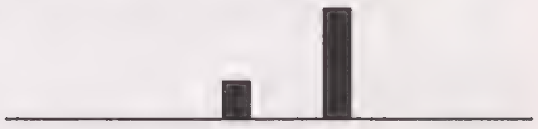
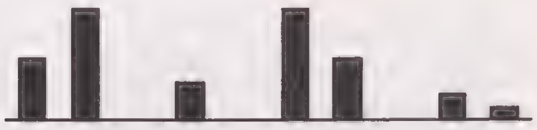
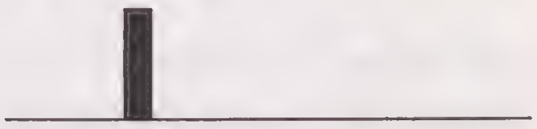
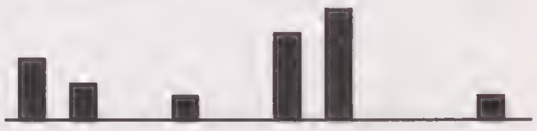
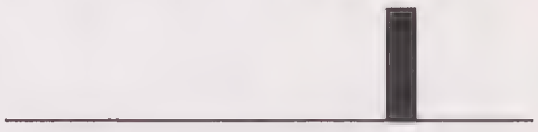
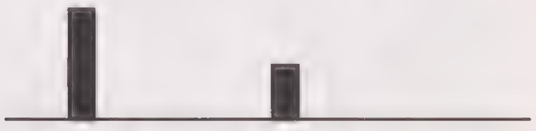
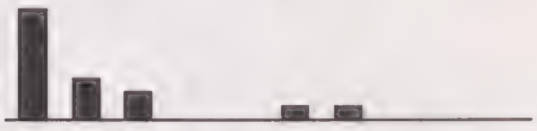
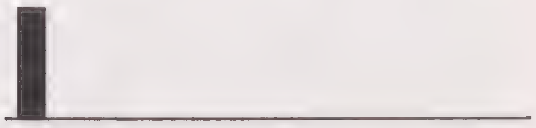
Taxon	Family	Total specimens	Total stations	Height (mm)	Diameter (mm)	H/D	MATURE MARANTACEAE OKOUME COL_SAVANNAS SAVANNAS ROCKY FOREST_FRAGM RIVER_DRIFTS ANTHROPIC GALLERY
Ptychotrema (Ennea) sp. 5	Streptaxidae	1	1	3.5	1.6	2.18	
Ptychotrema (Ennea) sp. 6	Streptaxidae	4	1	3.0	1.7	1.76	
Ptychotrema (Ennea) cf. *sylvatica* Pilsbry, 1919	Streptaxidae	1	1	3.2	1.3	2.46	
Marconia sp. 1	Streptaxidae	2	1	9.7	5.5	1.76	
Afropunctum cf. *seminium* (Morelet, 1873)	Punctidae	60	15	1	1.6	0.63	
Afropunctum sp. 2	Punctidae	10	4	1.3	2.1	0.62	
Kaliella cf. *barrakporensis* (L. Pfeiffer, 1852)	Euconulidae	145	33	2.8	2.9	0.97	
Afroguppya sp. 1	Euconulidae	1	1	1.7	1.9	0.89	
Afroguppya sp. 2	Euconulidae	153	28	0.8	1.4	0.57	
Trochozonites cf. *bifilaris* (Dohrn, 1878)	Urocyclidae	1	1	4	3.9	1.03	
Trochozonites sp. 4	Urocyclidae	10	3	4.6	4.2	1.10	
Trochozonites sp. 6	Urocyclidae	74	15	5.5	5.4	1.02	
Trochozonites sp. 8	Urocyclidae	12	1	7.0	7.4	0.95	

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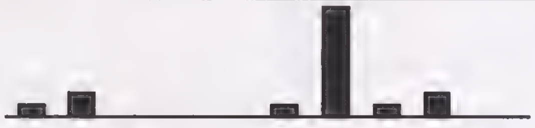
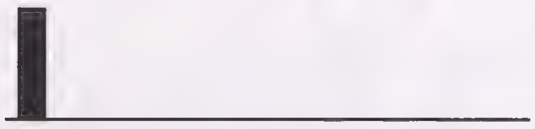
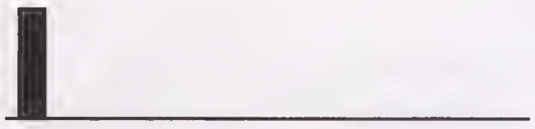
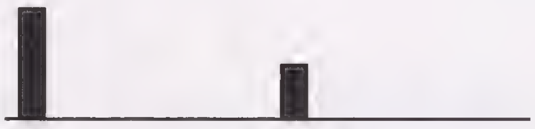
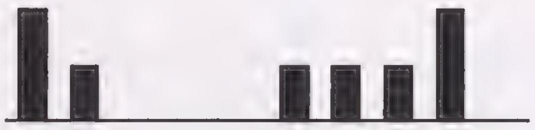
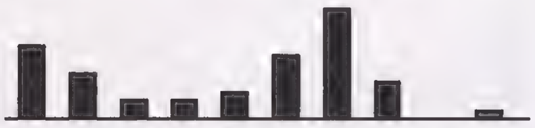
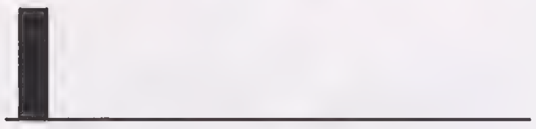
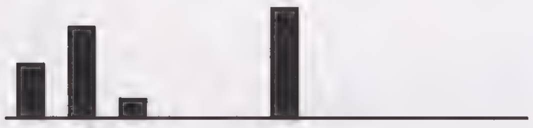
Taxon	Family	Total specimens	Total stations	Height (mm)	Diameter (mm)	H/D	MATURE MARANTACEAE OKOUME COL_SAVANNAS SAVANNAS ROCKY FOREST_FRAGM RIVER_DRIFTS ANTHROPIC GALLERY
Teleozonites adansoniae (Morelet, 1848)	Urocyclidae	51	15	5.5	5.4	1.02	
Teleozonites sp. 2	Urocyclidae	1	1	4.5	4.7	0.96	
Thapsia sp. 3	Urocyclidae	1	1	5.9	9.6	0.61	
Thapsia cf. *troglodytes* (Morelet, 1848)	Urocyclidae	3	3	26	15	1.73	
Gudeella sp. 1	Urocyclidae	57	7	3.6	6.0	0.60	
Gudeella sp. 2	Urocyclidae	279	44	4.5	8.1	0.56	
Zonitarion (Belonarion) n. sp. 1?	Gymnarionidae	4	3	-	-	-	
Zonitarion (Belonarion) n. sp. 2?	Gymnarionidae	32	15	-	-	-	

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SHELL PERMEABILITY AND DESICCATION PHYSIOLOGY OF THE FRESHWATER SNAIL *BULINUS* (*BULINUS*) *TROPICUS* (KRAUSS)

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ABSTRACT

The water permeability rate of the shell wall of *Bulinus tropicus* (Krauss) was determined with tritiated water to be 1.74 ±1.06 µl water per 24 h. The osmotic pressure of the haemolymph increased three-fold to 300 ± 16 mOsm l^{-1} after 60 days at 85% r.h. of the air. The [Na] of the snail tissues increased from 38 ± 9.0 mMol l^{-1} to 68 ± 11.0 mMol l^{-1} after 60 days at 85% r.h., but at 96% r.h. the increase changed from 40 ± 7.0 mMol l^{-1} to 55 ± 6.5 mMol l^{-1}. The [K] of the snail tissues increased from 30 ±10.1 mMol l^{-1} to 58 ± 11.2 mMol l^{-1} after 60 days at 85% r.h., but at 96% r.h. the increase changed from 30 ± mMol l^{-1} to 42 ± 10.2 mMol l^{-1}. The oxygen consumption rate (VO_2) decreased by 66% from 60.0 ±19.1µl O_2. g^{-1} snail. h^{-1} to 20.5 ± 16.0 µl O_2. g^{-1} snail. h^{-1} after 60 days at 96% and 85% r.h. In laboratory experiments without sunlight, snails were still alive after 5 months on mud. In field experiments conducted in direct sunlight on mud, snails were dead within 24 h. At r.h. of 96%, 92%, 85%, 74%, and 57% in the laboratory, the snail survival rate was respectively 40%, 40%, 42%, 22%, and 0% after 60 days. After 60 days at 96% r.h., the snail mass loss was 36%, but only 20% at r.h. of 74%.

Key words: aestivation, oxygen consumption rate, osmotic pressure, shell permeability, tissue electrolytes.

INTRODUCTION

Survival strategies of freshwater snail (intermediate) hosts of schistosomes during periods of dry conditions were described by Cort (1919), Barlow (1933, 1935), and Precht (1939). In a review, Appleton (1978) reported that all snail intermediate hosts for bilharziasis, such as *Bulinus* (*Bulinus*) *truncatus* and *Bulinus* (*Physopsis*) *africanus*, are able to tolerate desiccation in shaded temporary water bodies that gradually dried up. This also applies to *Bulinus tropicus*, an intermediate host for paramphistomiasis, which is a trematode that produces massive and acute infections in wild grazing domestic livestock in Africa (Brown, 1994). This usually occurs at the onset of the cooler winter season in combination with a high relative humidity layer created by mud in the close vicinity of the snail. Other factors, such as temperature, exposure time, snail size, and probably shell thickness, also play a role in determining desiccation tolerance (Gerard, 2001). Desiccation survival of freshwater snails are of epidemiological importance, because the infection can be carried over a dry period within the desiccated snail (Hira, 1968). The Bulininae, such as *Bulinus* (*Bulinus*) *tropicus* (Krauss) and *Bulinus* (*Physopsis*) *africanus* (Krauss), are better equipped to withstand desiccation in mud for up to one year compared to the Planorbinae, such as *Biomphalaria glabrata* and *Biomphalaria pfeifferi* (Appleton, 1978; Brown, 1994; Woolhouse & Taylor, 1990). Dormant land snails may survive for six years in museum collections without food or water (Comfort, 1957; Machin, 1967). An eco-physiological study (Schmidt-Nielsen et al., 1971) on a desert pulmonate snail (*Sphincterochila boissieri*) showed that snails can survive temperatures between 50°C and 55°C with a water loss rate of 0.5 µl per day for a 4 gram snail and a mean oxygen consumption rate of 15.63 µl O_2. h^{-1} for a 4 gram snail at 35°C but only 2.62 µl O_2. h^{-1} at 15°C.

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The African bulinids have been successfully induced to aestivate in the laboratory (Annecke & Peacock,1951; Cridland, 1967; Coles, 1969; Heeg, 1977) and in field experiments (Barlow, 1935; Coles, 1969; Shiff, 1964), but are also able to survive seasonal drying up of their natural habitats (Appleton, 1978). The field observations indicated that the snails survive better when the relative humidity is high. Field data (Shiff, 1964), however, gave no precise information as to what the tolerance limits in time were for snails exposed at precisely known different humidities. Laboratory studies by Vianey-Liaud & Lancastre (1986a, b) on adult and juvenile *Biomphalaria glabrata* showed that survival over long (16 weeks) periods occurred only when the atmosphere was saturated. Different terms have been used to describe animals exposed to dehydration conditions, which may finally result in anhydrobiosis (Stiglingh & Van Eeden, 1977), cryptobiosis, anabiosis, or anoxybiosis (Keilin, 1959; Clegg, 2001). Heeg (1977) and Brown (1994) uses the term aestivation to describe physiological quiescence, desiccation, and dormancy in freshwater snails kept out of water on a surface of damp river sand or mud.

Except for the work by Heeg (1975, 1977) on the oxygen consumption rate of *Bulinus* (*Physopsis*) *africanus* and Von Brand et al. (1957) on the physiology of *Biomphalaria glabrata* during aestivation, very little is known about the physiology and the water permeability of shells on freshwater snails during conditions of desiccation.

In this laboratory study, we hypothesized that the shell wall of *Bulinus* (*Bulinus*) *tropicus* is permeable to water, resulting in the diminution of body mass and oxygen consumption rate and thus causing an increase in haemolymph osmotic pressure and tissue sodium and potassium concentration. Radio-active labelled water (^3H_2O) was used to quantitatively determine shell permeability. The survival time of snails exposed at pre-determined humidities were also measured.

MATERIAL AND METHODS

Fifty full grown snails (9–10 mm) were collected at a gravel pit about 5 km from Potchefstroom adjacent to the Potchefstroom-Parys road (26°43.954'S, 27°8.109'E). This water body usually dries up at the end of the summer rain season. Another group of about 20 snails, already in a desiccated condition, was also collected. In the laboratory, the desiccated snails were placed in a breeding aquarium. After about one hour, they were actively crawling and feeding, and after five days in captivity they laid eggs. The F_1-generations of the large snail group and the desiccated field snails were used for the experiments and cultured in a aquarium unit with 34 litre capacity (De Kock & Van Eeden, 1986). The 34 litre capacity aquarium unit, consisting of a stainless steel container suspended inside a stainless water bath, was used. Polyvinyl chloride culture baskets, 15 cm in diameter and 11 cm in height with bottoms of fine Perlon gauze with a mesh size of 45 µm, were suspended in the aquarium water kept at 26°C. Snails were kept, fed (Tetramin Conditioning Food, Tetra-Werke, Germany), and reared in these baskets. Borehole water with water hardness of 193 mg l^{-1} (as carbonate) with concentrations of Ca, Mg, and bicarbonate of 1.98, 1.97, and 2.0 mMol l^{-1} respectively at a pH of 8.2, analysed by Midvaal Water Company (Accredited Lab. No T0132), was used. The culture water from the biological filter and activated charcoal tanks were supplied by gravity feed through small bore piping to each basket from an overhead reservoir.

Before each group of 60 adult aquarium snails, earmarked for the desiccation experiments, were exposed to either 92%, 85%, 74%, or 57% relative humidity (r.h.) in air at 15°C they were kept at 96% r.h. for one week at 15°C. To do this, each snail from a group was firstly placed in a small perforated Perspex weighing vial provided with a handle. The Perspex vial, with a known mass, was then hung from a hook, cemented with epoxy resin (Araldite) onto the underside of the glass lid of an 80 ml glass weighing vial. The relative humidity of the air in each of the sealed glass weighing vials was established with saturated salts of potassium sulphate (92%), zinc sulphate (85%), sodium chloride (74%), and sodium bromide (57%) (Olivier & Barbosa, 1958; Von Brand et al., 1957). Three ml of the saturated salt solutions were placed in each glass vial. For 96% r.h., filter paper saturated with water was used. The percentage mass decrease and survival rate of each snail, exposed to the different desiccation conditions, were determined every ten days for a period of 60 days. To establish death, each snail's foot surface, under a stereo-microscope, was stimulated with a needle. If no withdrawal response of the footpad was observed at 16 times magnification, the snail was considered dead. The remaining snails that survived the 60 day exposure periods recuperated fully when placed in aquarium water.

To determine the osmotic pressure of the haemolymph samples and the Na and K concentrations in snail tissue, samples were individually collected from snails after 15, 27, 45, and 60 days. Haemolymph was collected using the Bekius-effect (Lever & Bekius, 1965; S-Róza & Sakarov, 1989).

For the osmotic pressure and the sodium and potassium measurements, 60 snails for each measurement were respectively subjected to each of the different relative humidities. The osmotic pressure of the haemolymph in individual snails was determined with the aid of a nanoliter osmometer (Model 830, Fiske, USA). In the 12 wells of the osmometer, six wells were used for the standard (100 m Osm.kg^{-1} water), and the other six wells used for three samples from each of two snails.

To determine the potassium and sodium concentration of the snail tissue, individual snails were placed inside a dry test tube and killed by dipping part of the test tube in boiling water for 20 seconds. The snail was taken out of the test tube, and the dead body mass pulled out of the shell over a piece of aluminium foil. The wet body mass with its adherent body fluids was dried overnight at 65°C. This method was also used to determine the dried body mass, as a percentage of the total snail mass. After determination of the dried body mass the total tissue water was calculated by subtraction and the Na and K determined with a flame photometer (EEL 227 integrating flame photometer, UK), according to Van Aardt & Coertze (1981). For the osmotic and tissue potsssium/sodium measurements, at least seven snails per time interval were sacrificed for the analyses. The oxygen consumption rate (VO_2) was measured with a 14-flask Gilson-constant pressure manometric respirometer (Model GR 14, Gilson Medical Electronics, USA). The VO_2 of 20 control snails was measured in water using a Warburg-flask without a sidearm and the centre well filled with 10% KOH (Umbreit et al., 1972). The VO_2 of the experimental snails was measured at 96% and 85% r.h. in the air using 20 snails for each r.h. To do this, the bottom of the respiratory flasks were covered with 5 ml water (96% r.h.) or a saturated 5 ml solution of $ZnSO_4$ (85% r.h.) (Von Brand et al. 1957). On top of the empty centre well of the respiratory flask, each desiccated snail was kept in a conically shaped polyethylene mesh. The side arm of the flask contained a strip of filter paper dipped into 10% KOH. All measurements were made at 15°C and the VO_2 expressed in µl oxygen per gram live snail mass (with shell) per h. After the VO_2 measurements, the snails were transferred back to the glass vial set up for the different relative humidities. Sodium and potassium in the tissues as well as the osmotic pressure of the haemolymph and the VO_2 were measured for snails exposed in air only at 96% and 85% r.h.

To determine the permeability of the shell for tritiated water (^3H_2O), 20 adult snails were killed in boiled water. The freshly cleaned shells were dried for two h at 65°C. Under a stereo microscope, the mouth opening of each shell was heat sealed with a 1.5–2.0 mm thick layer of a aquarium sealant wax (Hykro Leak Stopper, Hykro, Denmark) using a hot spatula. Tritiated water (30µl) with an activity of 3.7 x 10^4 Bq per 50 µl was injected with a Hamilton syringe (Hamilton Co, USA) through the wax layer into the shell and the needle opening hot sealed. The fixed Hamilton needle was provided with a side opening near the needle tip to be able to prevent needle blockage during the injection. Each sealed shell was then placed into the Perspex vial, and hung in the 80 ml capacity sealed glass vial at a r.h of 96% in air for 24 h as described above. After the 24 h exposure period, a 2 ml sample of the water at the bottom of the 80 ml capacity glass vial was collected and counted for radioactivity (Van Aardt & Coertze, 1981). The amount of water that permeated through the shell wall after 24 h was determined by a radioisotope dilution method (Morris & Van Aardt, 1998). To detect possible leakage of the wax sealed shell mouth and to determine the permeability of the shell to high molecular substances, 30 µl radioactive inulin (Phelps, 1965), labelled with ^{14}C (4.1 x 10^4 Bq per 50 µl) was injected into another 20 sealed shells. The radioactive inulin solution sampled from the water at the bottom of the vial was counted in the same manner as described for tritiated water. As controls (in place of *B. tropicus* shells), 20 mini glass vials with 500 µl capacity was used. A 2 mm thick seal of the aquarium sealant wax was melted over the mini vial mouth as described for empty snail shells. A solution of 30 µl radioactive inulin was then injected and the vial mouth sealed and treated in the same manner described for the sealed empty shells.

Ten shells from the field population at Potchefstroom and the F_1-generation in the laboratory were cleaned and the thickness of the shell wall measured at the mouth opening by a digital calliper (Mitutoyo, Model CD-8; 0.01 mm). Ten shells from a soft water area (Rietfontein Dam, Pretoria) were also measured for shell thickness. For each population the carbonate hardness of the water was analysed by a Merck Aquamerck Analysis Kit 8048. For the laboratory experiments, all the measurements on snails were done without exposure to sunlight. To find out if non-sunlight conditions could influence the desiccation physiology of laboratory snails two field experiments, with and without sunlight was undertaken. Twenty specimens of *B. tropicus* were, for each experiment placed in a glass container (7 cm wide and 5 cm high) filled with 3 cm of soft mud (obtained from the gravel pit) covered by one cm of water to simulate field conditions (Cridland, 1967). The glass vials were placed into moist soil of 5 cm depth. Air temperature, in sunlight, was 25–27°C. Without sunlight, snails were kept in the same glass containers, with mud and water at 15–20°C.

RESULTS

For the experiments we used F_1-generation snails bred in the laboratory and not snails collected in the field. It was shown (Heeg, 1975) that field snails kept in aquarium water are subjected to physiological shock resulting in high mortalities before starting the desiccation experiments. In the first field experiment, with direct sunlight exposure, all the water evaporated within 5 to 7 days, and the snails were dead within 24 h. No attempt was made by the snails to dig or crawl into the mud. In the second field experiment without sunlight, snails were still alive after 5 months. The snails crawled into the mud with only a small part of the shell exposed with hardened mud sealing off the shell opening. Specimens of *B. tropicus* kept in air at all the five different relative humidities in the sealed glass containers were deeply contracted into the shell with the foot surface covered with what appears to be a thin layer of dried mucus.

The water carbonate hardness for the Potchefstroom habitat was 193 mg l^{-1} and for the Rietvlei Dam 103 mg l^{-1}. The mean shell

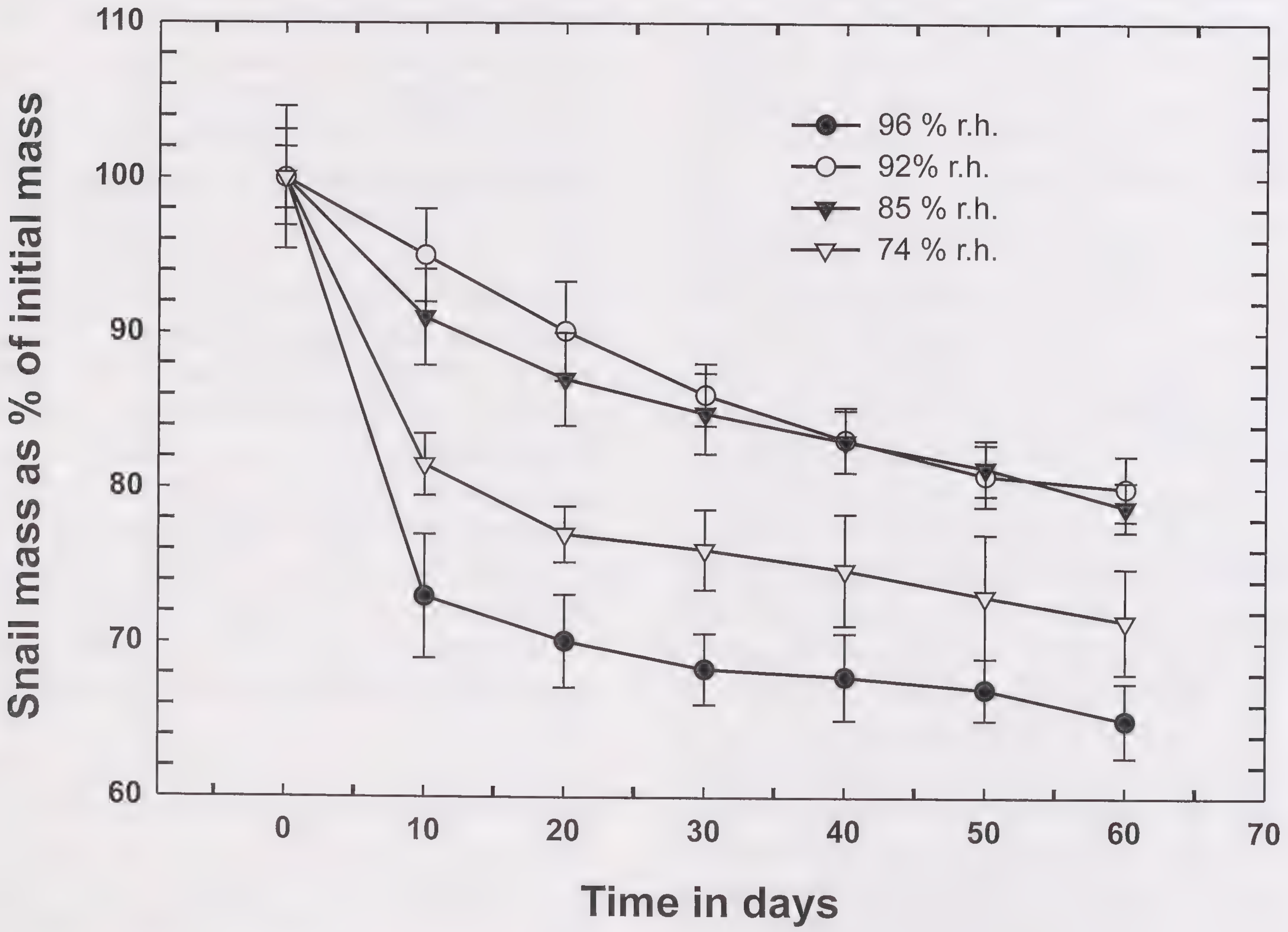

FIG. 1. Percentage decrease in snail mass during the 60 days exposure to different relative humidities (r.h.). Sixty snails were used for each r.h. Error bars denote the standard deviation from the mean.

thickness of the snails at the Potchefstroom population living at the gravel pit in hard water together with snails reared in the laboratory was 95.0 ± 2.0 µm and the shells from the softwater habitat at Rietvlei Dam was 27 ± 1.0 µm.

In general, the largest decrease of nearly 30% in mass, for the five different r.h. investigated, occurred within the first 15 days for the 96% and 74% r.h. exposed snails. After this period, the snail mass decreased, on average less than 10% for the next 45 days when exposed to the different r.h. (Fig. 1).

After 60 days at 96%, 92%, 85%, 74%, and 57% r.h. exposure, the percentage survival was 40%, 40%, 42%, 22%, and 0% snails, respectively. Between 37% and 42% of the snails exposed to 96%, 92%, and 85% r.h were still alive after 60 days, but at 74% r.h., 78% were dead, and at 57% r.h., all were dead 16 days after the onset of the experiment.

Sodium concentration in the snail tissue increased 79% to 68 mMol l^{-1} for snails exposed at 85% r.h., and 37.5% to 55 mMol l^{-1} at a r.h. of 96% for 60 days. For tissue potassium, the increase was 33% from 30 to 42 mMol l^{-1} at 96% r.h., and 83% from 30 to 58 mMol l^{-1} at 85% r.h.% (Fig. 2).

A threefold increase in the haemolymph osmotic pressure (Fig. 3) from 100 to 300 mOsm l^{-1} water was found for snails at 85% r.h. exposed for 60 days. For 96% r.h., exposed snails the increase was nearly two and a half times above the normal osmotic pressure value of 100 mOsm l^{-1} water.

From day 1 to day 60, the VO_2 decreased by 66% from 60 microlitres per gram snail (including the shell) per h to 20.5 microlitres per gram snail per h (Fig. 4). If the VO_2 is expressed per mg dried body mass (without the shell) (Van Aardt et al., 2003), it decreases from 0.627 µl O_2 mg^{-1} dried body mass hr^{-1} to 0.209 µl O_2 mg^{-1} dried body mass hr^{-1}. The VO_2 expressed in these units allows physiologists to compare oxygen consumption rates between pulmonates with and without shells. The decrease in the amount of oxygen consumed measured for 96% and 85% r.h. exposed snails after 60 days are the same (Fig. 4). Large variations in VO_2, however, were encountered between individual snails.

The tritiated water, as a percentage of the amount injected (30 µl) escaping through the shell wall, was 5.79% (Table 1), or 1.74 µl in 24 h. It was assumed that a snail with a snail

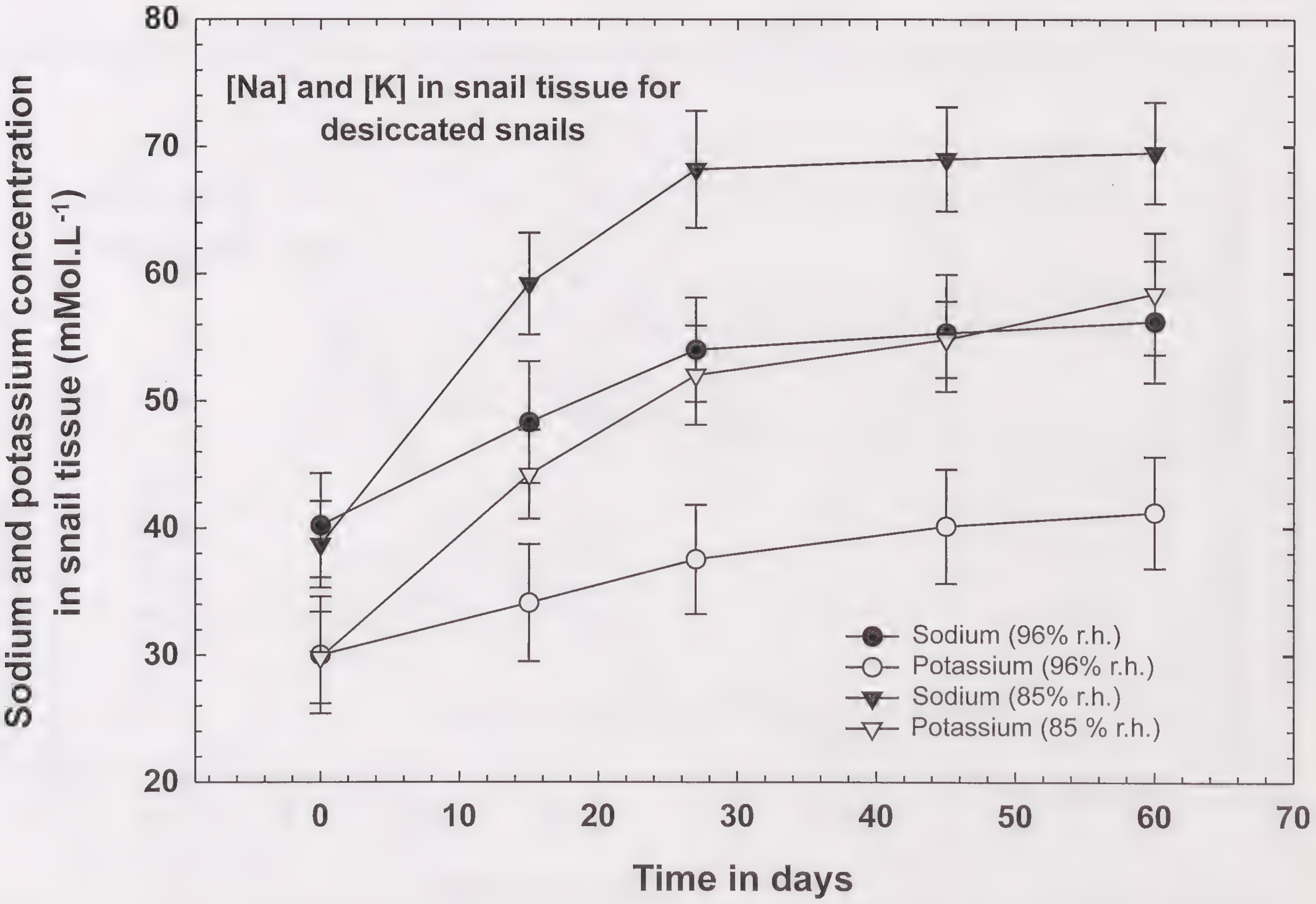

FIG. 2. The sodium and potassium concentrations in the tissues of snails exposed for 60 days at different relative humidities (r.h.).The number of snails alive after the exposure was 7, 7, 4, and 4 specimens for each r.h. Sixty snails were used for each r.h. Error bars denote the standard deviation from the mean.

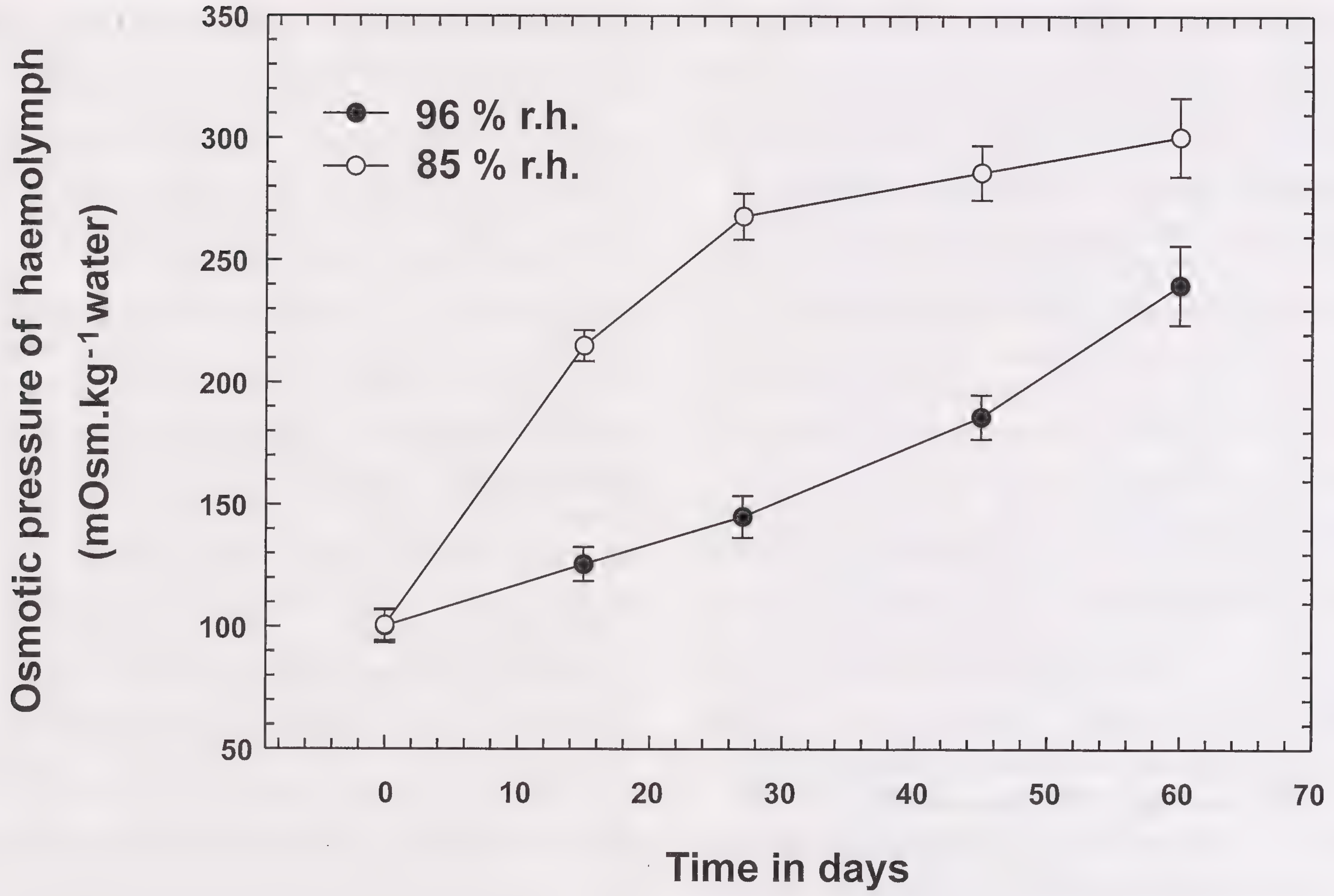

FIG. 3. The osmotic pressure of the haemolymph for each group of 40 snails exposed to 96% or 85% relative humidity. At least seven snails were used for each determination. Error bars denote the standard deviation from the mean. The number of snails alive after the exposure period was 5 specimens for each r.h.

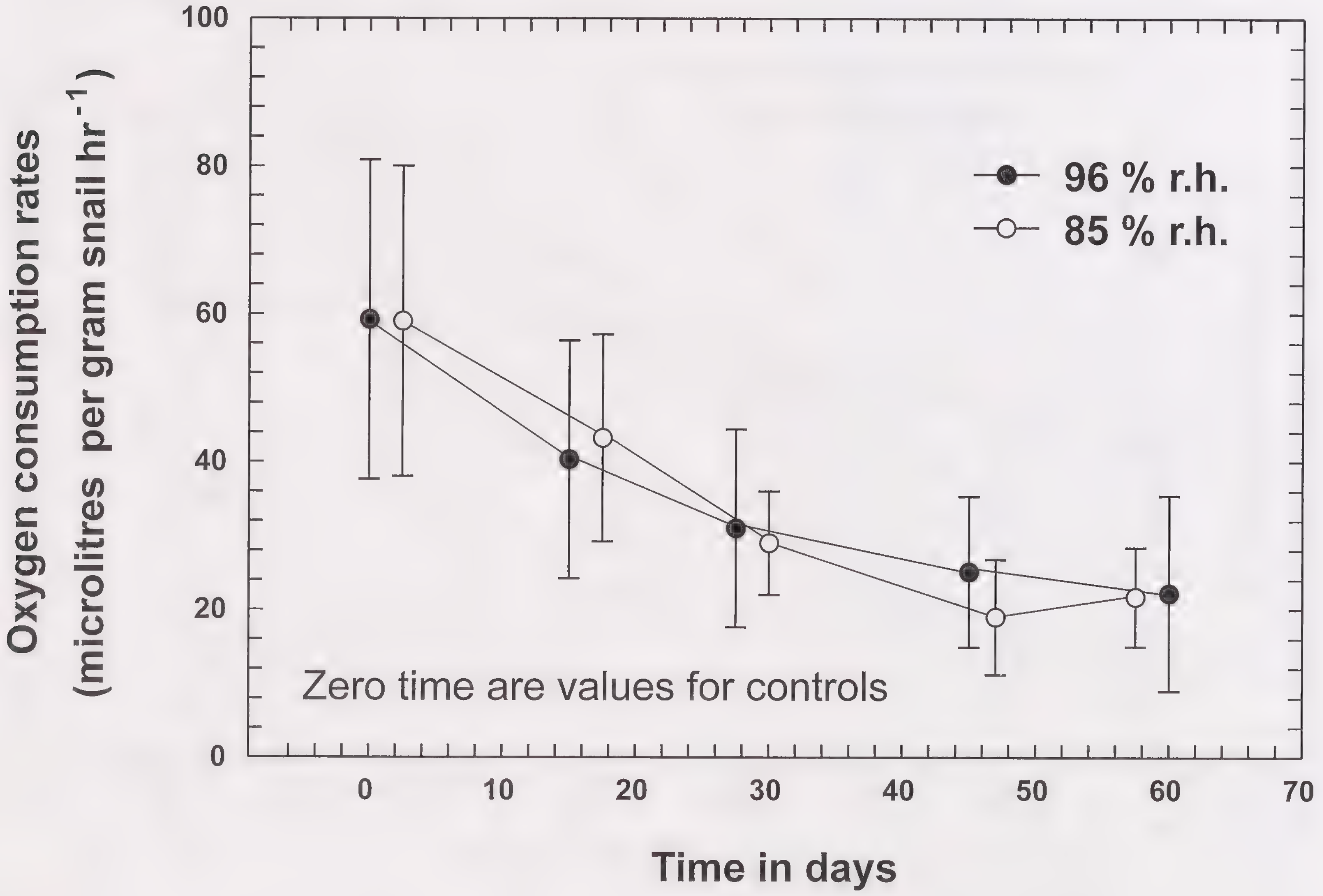

FIG. 4. Oxygen consumption rates at 15°C for aestivated snails at 96% and 85% r.h. over a period of 60 days.

TABLE 1. Tritiated water and Inulin-^{14}C found in the water of the 80 ml sealed glass vials 24 h after injection into the shells of *Bulinus tropicus* and the mini glass vials. Dpm: disintegrations per minute; ±: Standard deviation from the mean; *Data not used in the calculations (see text).

Shell no	Dpm of ^3H_2O found in the water	% of ^3H_2O found in the water	Shell no	Dpm of inulin found in the water	% of Inulin found in the water	Mini vial no	Dpm of inulin found in the water	% of inulin found in the water
1	2273	0.90	1	73	0.13	1	0	0
2	9783	3.86	2	233	0.43	2	0	0
3	12263	4.84	3	8603*	15.70*	3	0	0
4	78460	30.94*	4	463	0.44	4	0	0
5	86573	34.13*	5	133	0.24	5	4700*	8.58*
6	7398	2.92	6	16460*	30.03*	6	328	0.60
7	25803	10.17	7	0	0	7	153	0.28
8	7548	2.98	8	135	0.25	8	0	0
9	16936	6.69	9	5	0.01	9	0	0
10	28218	11.13	10	0	0	10	0	0
11	21033	8.29	11	0	0	11	270	0.49
12	19238	7.59	12	108	0.2	12	5	0.01
13	2640	1.04	13	325	0.59	13	5	0.01
14	2433	1.35	14	575	1.05	14	3	0.01
15	14675	5.79	15	183	0.33	15	10	0.01
16	16130	6.36	16	0	0	16	35	0.02
17	15888	6.26	17	123	0.22	17	15	0.01
18	25760	8.16	-	-	-	18	43	0.02
19	24850	9.10	19	11	0.02	19	5	0.01
						20	33	0.02
		5.79 ± 3.20			0.37 ± 0.29			0.12 ± 0.20

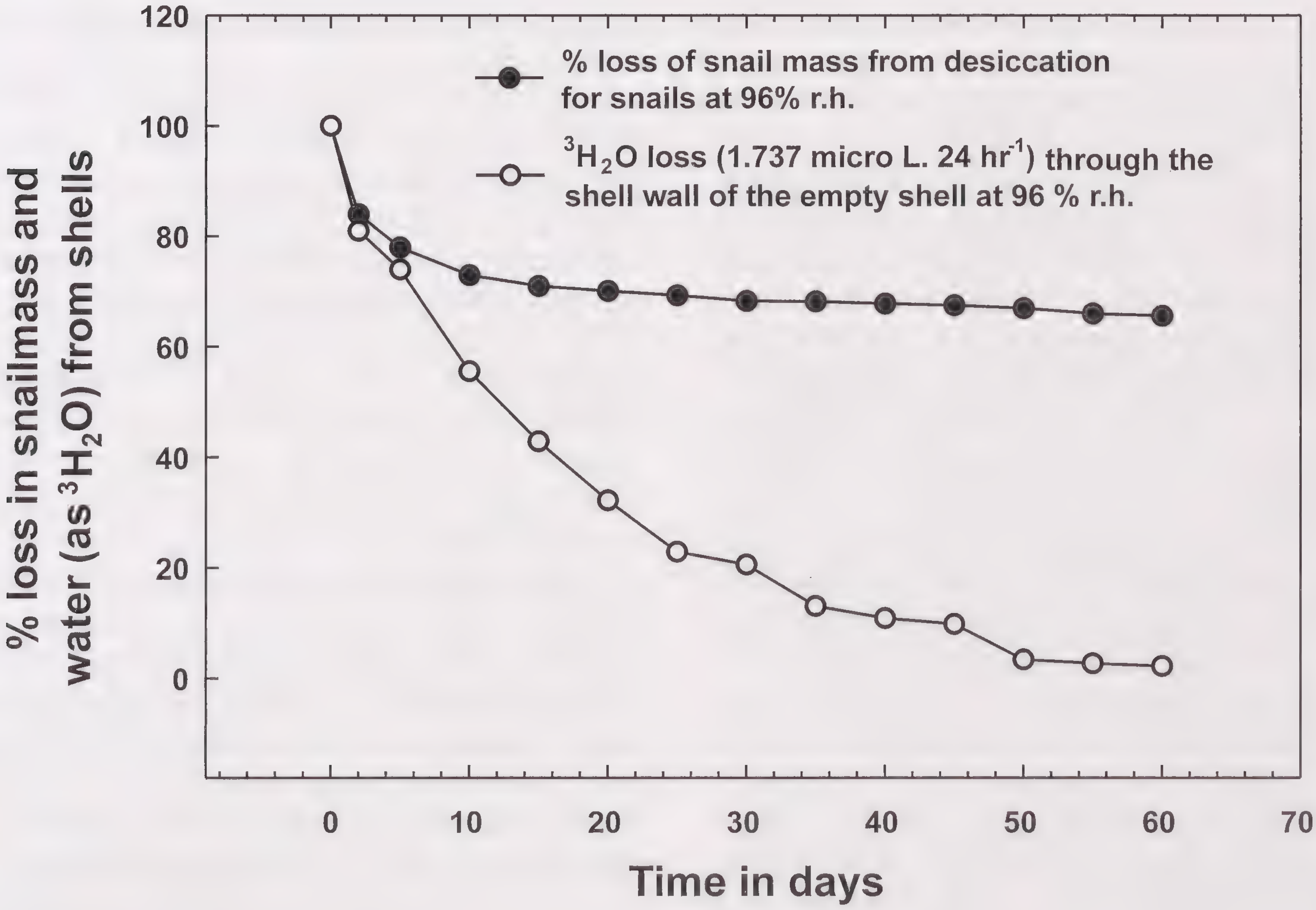

FIG. 5. Measured loss of snail mass during desiccation and calculated loss of tritiated water through the wall of empty sealed shells after 60 days.

body mass of 0.0917 gram would have a shell volume of 91.7 µl. With a daily loss of 1.74 µl water, the shell would be empty of water after about 60 days (Fig. 5). When tritiated water values are compared with the high molecular mass compound, inulin, only 0.37% of inulin was found in the water of the sealed glass vial and only 0.12% of inulin found in the water of the sealed 500 µl mini glass vial. The very high inulin radioactive values found in the water of the inulin injected shells (Table 1, shown with an asterisk) are due to leakages through the sealant, because similar leakages were found with the sealed mini glass vials when injected with inulin. Therefore, data annotated with asteriks were not used to evaluate the results. In Figure 5, the percentage loss in snail mass is compared with the calculated cumulative tritiated water loss through the shell wall kept at 96% r.h. The results indicate that the tritiated water injected into the empty shell through the sealed off shell mouth was all lost after 60 days. In comparison, the water component in the desiccated snail, withdrawn inside the shell, decreased by only about 40% during the 60 days exposure to 96% r.h.

DISCUSSION

Our measurements with tritiated water clearly show that the shell wall of *Bulinus* (*Bulinus*) *tropicus* is permeable to water. This cannot be ascribed to leakages of ^3H_2O through the sealant that covers the shell mouth or by other phenomena. So far as we know, this is the first attempt to measure the permeability of the shell wall for water of a freshwater snail. Compared with molluscs with an outer layer of periostracum, middle prismatic layer and a thick inner nacreous layer of more than 1 mm in thickness (Nakahara & Bevelander, 1971), the shell walls of the African basommatophorans are relatively thin. The ultrastructure and cytochemistry of the shell wall in a related basommatophoran, *Biomphalaria glabrata* (Peters, 1972; Bielefeld et al., 1993a) consists of an 1.6 µm thick outer layer or periostracum composed almost entirely of scleroprotein together with some bound carbohydrates and also chitin (Hunt & Oates, 1978). The inorganic part of the shell consists mainly of both organic matrix materials and $CaCO_3$ as the inorganic crystalline substance (Bielefeld et al., 1993b). Nothing is known about the water permeability ability of these two layers. In *B. glabrata* reared in the laboratory, the total shell wall is 140 ± 0.02 µm thick (Van Aardt, personal observation) compared to 95 µm for *B. tropicus.* The shell walls of *B. tropicus* grown in the Rietvlei Dam with its soft water are about three times thinner compared with snails grown in the two hard water habitats at Potchefstroom. From the permeability data, it can be deduced that the thicker shell walls of the Potchefstroom populations, growing in hard water, should be less permeable to water and thus more resistant to conditions of desiccation. It is interesting to note (Fig. 5) that less water is lost in the living snail during aestivation compared with the empty shell, partly filled with radioactive water. A possible explanation is that the peripheral snail tissues, especially the mantle and muscular foot, are effective barriers that prevent water evaporation during the tight contraction into the shell of the soft body parts.

The relative large loss in water for the 96% r.h. exposed snails compared to the 85% and 92% r.h. exposed snails (Fig. 1) could be explained by the activity of the snails during the exposure period. The soft body parts of these snails are observed to periodically extend out from their shells and in the process expose the wet body surface to evaporative water loss into the aerial space of the sealed container.

The oxygen uptake rates for *B. tropicus* are in good agreement with those reported for other basommatophorans (Von Brand et al., 1957; Van Aardt et al., 2003). After 60 days of desiccation at 15°C, the VO_2 was 0.209 ± 0.07 µl O_2 mg^{-1} dried snail hr^{-1}, which is nearly the same as the 0.33 µl O_2 per mg dried snail tissue per h found for *Bulinus* (*Physopsis*) *africanus* after 21 days in aestivation at 15°C (Heeg, 1977). Evidence from the literature on VO_2 data and metabolite levels (Schmidt-Nielsen et al., 1971; Heeg, 1977) suggests that during desiccation snails use stored reserves sparingly even at high temperatures, with lipids, carbohydrates, and proteins metabolised in equal amounts.

The decrease in respiration rate and body mass, with a concomitant increase of the haemolymph [Na], [K] and osmotic pressure, during the 60 days exposure of *B. tropicus* in air is indicative of the ability of *B.* (*B.*) *tropicus* to survive conditions of desiccation. Most species of *Bulinus* living in African water bodies have the ability to aestivate, which may have contributed to their success as carriers of schistosomes and other waterborne parasites

(Brown, 1994). Our oxygen consumption rate measurements on *B. tropicus* are in good agreement with those of Coles (1969) on *Bulinus nasutus*, and our snail mass decrease determinations agree with those of Oliver & Barbosa (1958) on *B. glabrata* using field collected snails. However, the physiology of *B.* (*B.*) *tropicus* during desiccation indicates that the adaptations these snails made cannot be compared to true cryptobiotic or anhydrobiotic animals, such as nematodes, copepods, tardigrades, and brine shrimps, for which oxygen uptake rates are not measurable and body water is almost totally lost (Clegg, 2001).

During the seven days exposure to 96% r.h. in air, all the snail groups were active and showed crawling movements of the exposed footpad. After this period, movements were only observed for snails kept at the 92% r.h. for day 8 to 9, after which deep withdrawal into the shells occurred. This behaviour was also found for *Pila virens* (Meenakshi, 1956), *Pomacea lineata, P. depressa* (Little, 1967), and for *Bulinus* (*Physopsis*) *africanus* (Heeg, 1977). Brown (1994) classified *Bulinus tropicus* as a good aestivator, together with five other bulinids living in ephemeral waters that may not be defined as aquatic habitats during the dry season. At 12–14% relative humidity exposure of *Heliosoma trivolvis,* Gallo et al. (1984) found a LD_{50} at 20–22°C after 45 h exposure, whilst the haemolymph osmolality increased from 99 $mOsm.kg^{-1}$ H_2O to 173 $mOm.kg^{-1}$ H_2O.

The high rate of water loss for the five different r.h. groups, kept at 96% r.h. during the first 10 days, could be explained by the relatively high activity of the snails resulting in the loss of water between the shell and snail body. This was followed by a much slower rate of water loss for snails kept from day 10 at 92%, 85%, 74%, and 57% r.h. (Fig.1). The water loss originates mainly from body water inside the snail (Komiya & Hasimoto, 1958).

The three-fold increase of the of the haemolymph osmotic pressure and the concomitant increase of the tissue [Na] and [K], together with a loss in body mass, indicates water loss as the major factor in the desiccation condition of the snail. However, little is known how extensively freshwater snails can tolerate increases of electrolytes in the tissues or in the haemolymph . Water loss at 12–14% r.h results in an increase of the haemolymph osmotic pressure in *Heliosoma trivolvis* from 100 mOsm l^{-1} to 173 ± 10 mOsm l^{-1} within 18 h (Gallo et al., 1984).

Our results thus show that *B. tropicus* can adapt physiologically to desiccation in the laboratory and most probably in the field, but it is still uncertain whether biochemical and biophysical mechanisms are involved in freshwater snails in which polyhydroxy compounds such as trehalose play a major part in well-adapted anhydrobiotic organisms (Clegg, 2001). For freshwater snails, induction to the desiccation process, is subject to low temperatures, initial high r.h. with no surrounding water and applicable to full grown snails. *Bulimus* (*B.*) *tropicus* and *B. truncatus* are more resistant to desiccation than *Biomphalaria* species because of their mud burrowing ability (Ghandour, 1987). The water permeability of the shell wall in freshwater snails should be systematically investigated to establish the magnitude of this permeability. It is known that both genetic and mechanical factors are implicated in the ability of freshwater snails to survive out of water (Brown, 1994).

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THE TAXONOMIC POSITION OF *BENTHARCA XENOPHORICOLA* (KURODA, 1929) AND *BENTHARCA RUBROTINCTA* KURODA & HABE IN HABE, 1958 (BIVALVIA: ARCIDAE)

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ABSTRACT

Bentharca xenophoricola (Kuroda, 1929) and *Bentharca rubrotincta* Kuroda & Habe in Habe, 1958, from Japan are re-examined. Based on comparison with the material of *Bentharca asperula* (Dall, 1881) and *Asperarca nodulosa* (Müller, 1776), the type species of *Bentharca* Verrill & Bush, 1898, and *Asperarca* Sacco, 1898, respectively, as well as on the description, pictures, and photos of *Deltaodon tugelae* Barnard, 1962, the type species of *Deltaodon* Barnard, 1962, the following new combinations are suggested: *Asperarca xenophoricola* (Kuroda, 1930) and *Deltaodon rubrotincta* (Kuroda & Habe in Habe, 1958). *Asperarca xenophoricola* has an elongate, inflated, thick shell, with a weakly hirsute periostracum, commarginal lamellate ridges made nodulose by the closely spaced, high radial riblets, and narrow, slightly curved hinge plate, with teeth divided into anterior and posterior sets by a narrow edentulous gap. The anterior set of this species has 7–14 long teeth; the posterior set 6–9 oblique teeth that diverge to a varying degree. This species occurs off Japan, China, and southeastern Australia at depths from 50 to 500 m. *Deltaodon rubrotincta* has a high, moderately inflated, thick, reddish-brown shell, with white rays and spots, commarginal lamellate ridges and high, nodules, widely spaced radial ribs, a broad, strongly curved hinge plate, with teeth divided into anterior and posterior sets by a shallow, oblique groove. The anterior set of this species forms an oval group of 4–7 long, mostly curved, boomerang-shaped teeth. The posterior set has 9–18 long, oblique teeth that diverge to a varying degree. This species occurs off Japan and China at depths from 100 to 200 m. Expanded descriptions of these species are presented.

Key words: *Bentharca*, *Asperarca, Deltaodon,* systematics, morphology, distribution, northwestern Pacific.

INTRODUCTION

Bentharca Verrill & Bush, 1898, species are widely distributed in the world's oceans (Dall, 1881; Verrill & Bush, 1898; Knudsen, 1967, 1970; Oliver & Allen, 1980; Oliver & Cosel, 1993), including the North Pacific (Kuroda, 1930; Habe, 1958, 1977, 1981; Li, 1984; Habe et al., 1986; Hayami & Kase, 1993; Higo et al., 1999; Coan et al., 2000; Okutani, 2000). The shell morphology of most species of *Bentharca* has been described in detail (Dall, 1881; Knudsen, 1967, 1970; Oliver & Allen, 1980; Hayami & Kase, 1993; Coan et al., 2000). Exceptions are *Bentharca xenophoricola* (Kuroda, 1929) and *Bentharca rubrotincta* Kuroda & Habe in Habe, 1958. The descriptions of these species given in the literature are brief (especially for *B. xenophoricola*) and sometimes without illustrations (Kuroda, 1929, 1930; Habe, 1951, 1958, 1977; Li, 1984; Okutani, 2000). The present study of the shell morphology of these species and the representatives of other genera of the Arcidae has shown that *B. xenophoricola* and *B. rubrotincta* should be placed in the genera *Asperarca* Sacco, 1898, and *Deltaodon* Barnard, 1962, respectively. In this paper, I give expanded descriptions of *B. xenophoricola* and *B. rubrotincta*, supplemented by new data on their shell morphology that support the new combinations.

MATERIAL AND METHODS

Material Studied

The collections of the following taxa were used: *Bentharca asperula* (Dall, 1881) (LACM, MIMB, NMNZ, NMW, NSMT); *Asperarca nodulosa* (Müller, 1776) (NMW); *B. rubrotincta* (NSMT); *B. xenophoricola* (NMW); *Samacar strabo* (Hedley, 1915) (AMS); *Samacar pacifica* (Nomura & Zinbo, 1934) (NSMT, SBMNH); several species of *Acar* Gray, 1857; *Barbarca* Dall, Bartsch & Rehder, 1938; *Barbatia* Gray, 1842; *Bathyarca* Kobelt, 1891; *Coraliarca* Habe & Okutani, 1968; *Hawaiarca* Dall, Bartsch & Rehder, 1938; and *Mimarcaria* Iredale, 1939 (CAS, MIMB, NSMT, RBCM, SBMNH, UW, ZIN). *Bentharca asperula* from New Zealand was fixed and stored in 70% ethanol. All other material was stored dry.

Shell Measurements

Figure 1 shows the shell morphology measurements. Shell length (L), anterior end length (A), height (H), width (W), and interumbonal distance (L1) were measured for shell pairs and each valve. The ratios of these parameters to shell length (A/L, H/L, W/L, L1/L, respectively) were determined. Anterior and posterior teeth in each valve were also counted. Shell measurements were made using a caliper and an ocular micrometer with an accuracy of 0.1 mm.

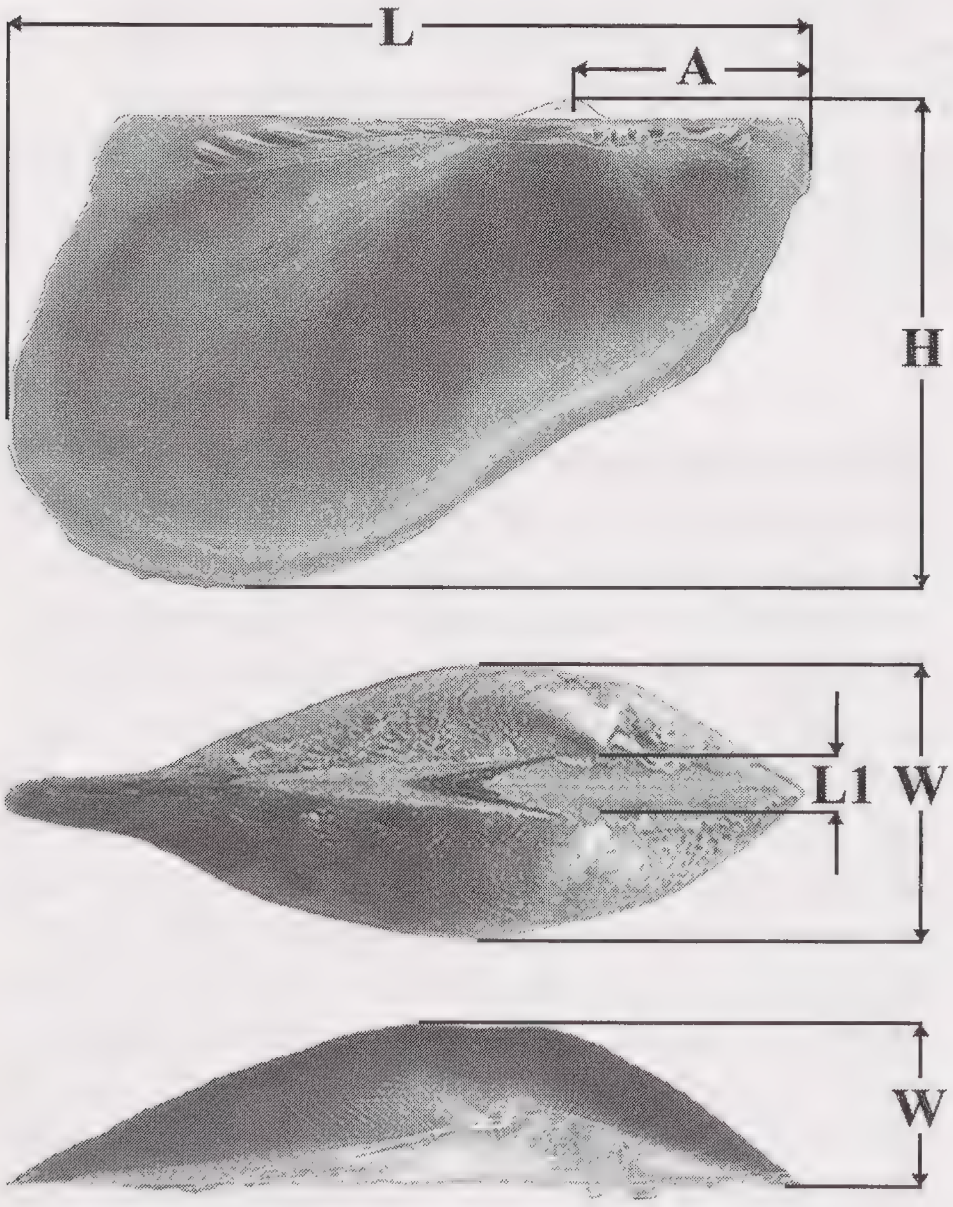


FIG. 1. Placement of shell measurements: L – length; H – height; A – anterior end length; W – width; L1 – interumbonal distance.

The following material was measured:

(1) twenty-four specimens and four left valves of *Bentharca asperula* from the Pacific and Atlantic oceans: North Pacific (NMW 1955.158.10248, 6 specimens); Tufts Abyssal Plain, North Pacific (LACM 1976-84.10, 1 specimen); Kermadec Ridge, South Pacific (NMNZ M.174251, 6 specimens); southwestern Africa, Atlantic (MIMB 12373 (donation from NMW), 10 specimens); Azores, North Atlantic (LACM 70-255.2, 1 specimen); Florida, North Atlantic (NMW 1955.158.10247, 4 left valves).

(2) ten specimens of *Asperarca nodulosa* from Norway, northeastern Atlantic (NMW 1955.158.10252, 6 specimens; NMW 1955.158.10253, 4 specimens).

(3) nine specimens of *Bentharca xenophoricola* from Japan, northwestern Pacific (NMW 1955.158.10256, 7 specimens; NMW 1955.158.10257, 2 specimens).

(4) six specimens of *Bentharca rubrotincta* from the Ryukyu Archipelago, Japan, northwestern Pacific (NSMT 51916).

Methods Used

For scanning electron microscopy, shells were cleaned of traces of soft tissues and periostracum in a strong commercial bleach, washed in distilled water, and dried. They were then mounted on aluminium stubs using adhesive tape and coated with gold for examination with a LEO 430.

Abbreviations

The following institutional abbreviations are used in the paper: AMS – Australian Museum, Sydney; CAS – California Academy of Sciences, San Francisco; IMB – Institute of Marine Biology, Russian Academy of Sciences, Vladivostok; LACM – Natural History Museum of Los Angeles County, Los Angeles; MIMB – Museum of the Institute of Marine Biology, Vladivostok; NMNZ – Museum of New Zealand Te Papa Tongarewa, Wellington; NMW – National Museums & Galleries of Wales, Cardiff; NSMT – National Science Museum, Tokyo; RBCM – Royal British Columbia Museum, Victoria; SAM – South African Museum, Cape

Town; SBMNH – Santa Barbara Museum of Natural History, Santa Barbara, California; UW – University of Washington, Seattle; ZIN – Zoological Institute, Russian Academy of Sciences, St. Petersburg.

SYSTEMATICS

Family Arcidae Lamarck, 1809
Genus *Asperarca* Sacco 1898

Type species

Arca nodulosa Müller, 1776, original designation by Sacco, 1898: 10.

Diagnosis

Shell small (< 15 mm), moderately thick to thick, moderately inflated, ovate-trapezoidal to subrectangular, equivalve or slightly inequivalve (left valve sometimes larger and more inflated), strongly inequilateral. Umbones strongly anterior, prosogyrate. Cardinal area wide. Shell surface with a byssal flexure more expressed in left valve. Sculpture of slightly imbricated commarginal lamellate ridges, made crenulate or nodulose by closely spaced, wide radial riblets. Periostracum thin, sometimes slightly hirsute, with short setae along posteroventral margin. Hinge plate narrow, slightly curved. Teeth divided into anterior and posterior sets by an edentulous gap; anterior teeth short, subvertical or oblique; posterior teeth longer, more oblique. Adductor scars slightly raised. Byssal sinus faint to moderate. Ligament external, opisthodetic.

Remarks

The genus *Asperarca* with the type species *A. nodulosa* was separated by Sacco (1898) when he studied *Acar* cf. *nodulosa* (Müller) and described its "varieties" *subandosa, perelliptica, carinatula*, and *subelliptica*. Subsequently, Newell (1969) synonymized it with *Barbatia* Gray, 1842, and until recently *A. nodulosa* was placed to *Bentharca* (Oliver & Allen, 1980; Oliver & Cosel, 1993). However, La Perna (1998) showed that *A. nodulosa*, together with a few Miocene species and two small Mediterranean species, form a distinct group to which the genus name *Asperarca* should be applied. Based on analysis of Noda's work (Noda, 1988), La Perna (1998) also noted that in shell morphology *B. xenophoricola* is similar to *Asperarca* rather than to *Bentharca*. Besides the morphological similarity, the bathymetric range of *B. xenophoricola* is in accord with the ecological range of *Asperarca* (La Perna, 1998).

After examination of the shell morphology of *B. xenophoricola* and type species of the genera *Asperarca* and *Bentharca* – *A. nodulosa* and *B. asperula*, respectively (Figs. 2–42, Tables 1–5), it is clear that *B. xenophoricola* should be recognized as a species of *Asperarca. Bentharca xenophoricola* (Figs. 30–42, Tables 4–5) differs from *B. asperula* (Figs. 2–16, Tables 1–2) in having the more angular, elongate, inflated and thicker shell, a less expanded posterior shell end, posterior radial carina, stronger radial sculpture, weakly developed hirsute periostracum, narrow edentulous gap between the anterior and posterior sets of teeth. In shell morphology, *B. xenophoricola* is more similar to *A. nodulosa* (Figs. 17–29, Tables 1, 3), with which it shares the angular, elongate, inflated and thick shell, a less expanded posterior shell end, well-defined radial carina, strong radial sculpture, weakly developed hirsute periostracum, and a relatively narrow edentulous gap. Therefore, following the recommendation of La Perna (1998), *B. xenophoricola* is here assigned to *Asperarca.*

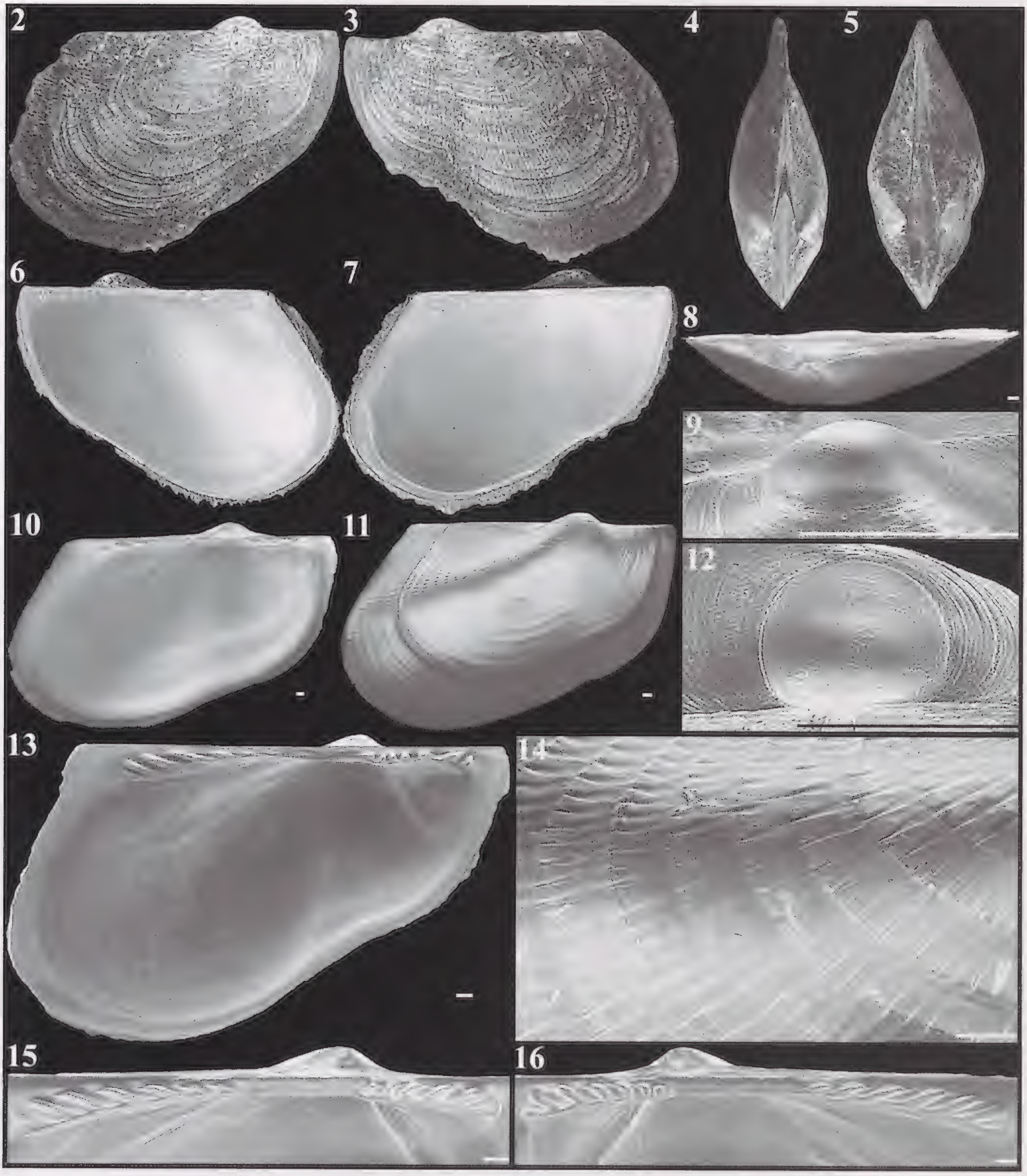
In addition to *B. xenophoricola*, four small species of *Bentharca* (shell length up to 4.0 mm) were recorded from off Japan. These are *Bentharca tenuis* Hayami & Kase, 1993; *Bentharca decorata* Hayami & Kase, 1993; *Bentharca irregularis* Hayami & Kase, 1993; and *Bentharca excavata* Hayami & Kase, 1993, described from the sublittoral caves of Ryukyu Islands (Hayami & Kase, 1993; Okutani, 2000). In shell morphology, these species are by far more similar to *Asperarca* than to *Bentharca*. The genus *Asperarca* now contains two small species (with a shell length up to 3.9 mm) described by La Perna (1998) from the Mediterranean Sea. Therefore, it is quite possible that following their detailed examination, four small species from coastal waters of Japan will also be placed in the genus *Asperarca*.

Asperarca xenophoricola (Kuroda, 1929)
(Figs. 30–42, Tables 4–5)

Bathyarca (?) *xenophoricola* Kuroda, 1929: 16, figs. 38–39.
Bathyarca (?) *xenophoricola* Kuroda – Kuroda, 1930: 33.

TABLE 1. Main morphological characters of the compared species. L – shell length; H – height; W – width; L1 – interumbonal distance.

Characters	*Bentharca asperula*	*Asperarca nodulosa*	*Asperarca xenophoricola*	*Deltaodon tugelae*	*Deltaodon rubrotincta*
Shall shape (H/L)	Trapezoidal, with strongly expanded posterior end; high (0.679)	Trapezoidal, elongate, with expanded posterior end; moderately high (0.613)	Trapezoidal, elongate, with expanded posterior end; moderately high (0.604)	Trapezoidal, with expanded posterior end; very high (0.764)	Trapezoidal, with strongly expanded posterior end, reddish brown with white rays and spots; high (0.667)
Valve (W/L)	Moderately inflated (0.379)	Very inflated (0.468)	Very inflated (0.444)	Moderately inflated (0.412)	Moderately inflated (0.373)
Interumbonal distance (L1/L)	Large (0.111)	Small (0.062)	Large (0.103)	Very small	Very small (0.023)
Sculpture	Wide, low commarginal ridges and wide, flat, closely spaced radial riblets	Wide, slightly imbricated, commarginal lamellate ridges and wide, high, closely spaced radial riblets	Wide, slightly imbricated, commarginal lamellate ridges, made nodulose by wide, high, closely spaced radial riblets	Wide, slightly imbricated, commarginal lamellate ridges and widely spaced radial riblets in middle part of shell	Wide, slightly imbricated, commarginal lamellate ridges and nodulose, widely spaced radial riblets of varying breadth
Periostracum	Thick, gray or brown, strongly hirsute; dense setae broad to narrow	Thin, pale gray, weakly hirsute, with thin, short setae	Thin, pale gray or pale brown, weakly hirsute, with thin, short setae	Thin, hairless	Thin, pale gray, weakly hirsute, with thin, short setae
Byssal flexure	Shallow	Very shallow	Deep	Absent in adult specimens	Shallow
Hinge plate	Subhorizontal, narrow	Slightly curved, narrow	Slightly curved, narrow	Curved, broad, strongly widening anteriorly and posteriorly	Curved, broad, strongly widening anteriorly and posteriorly
Edentulous gap	Wide, smooth	Narrow, smooth	Narrow, smooth	Very narrow, with shallow oblique groove	Very narrow, with shallow oblique groove
Hinge (number of teeth)	Anterior teeth short, subvertical (4–10); posterior teeth longer, oblique (4–8)	Anterior teeth short, oblique (4–6); posterior teeth longer, oblique (10–12)	Anterior teeth subvertical (7–14); posterior teeth oblique (6–9)	Anterior teeth long, curved, some bifid at their lower ends (8–9); posterior teeth long, oblique to subhorizontal (9–13)	Anterior teeth long, oblique, mostly curved, boomerang-shaped (4–7); posterior teeth long, oblique (9–18)



FIGS. 2–16. *Bentharca asperula.* FIGS. 2–3, 6–7: Exterior and interior views of right (length 10.1 mm) and left (length 10.3 mm) valves, southwestern Africa, 3975 m (MIMB 12373); FIG. 4: Dorsal view of both valves (shell length 8.8 mm), North Pacific (35°49'N, 179°57'W), 4605 m (NMW 1955.158.10248); FIG. 5: Dorsal view of both valves (shell length 8.9 mm), Kermadec Ridge (31°06.39'S, 179°02.05'W), New Zealand, 1522 m (NMNZ M. 174251); FIG. 8: Dorsal view of the left valve (MIMB 12373); FIGS. 9, 12: Prodissoconch of left valve (MIMB 12373); FIG. 10: Interior view of left valve without periostracum (MIMB 12373); FIGS. 11, 14: Exterior view and sculpture of right valve without periostracum (MIMB 12373); FIG. 13: Interior view of left valve (NMNZ M. 174251); FIGS. 15–16: Hinge of left and right valves (MIMB 12373). Scale bars = 300 µm.

Arca (*Bathyarca*) *xenophoricola* (Kuroda), 1929 – Nomura & Zinbo, 1934: 114.
Bentharca xenophoricola (Kuroda) – Habe, 1951: 33, figs. 45–46.

Type Locality

Kij Peninsula (central Honshu), 241 m (Kuroda, 1930; Higo et al., 1999).

Material Examined

Nine specimens from Kij, Japan, Northwest Pacific (NMW 1955.158.10256, 7 specimens; NMW 1955.158.10257, 2 specimens); 1 right and 1 left valves (AMS C 047185 together with *S. strabo*), 22 mi E of Narrabeen, N of Sydney, New South Wales, Australia (33°42'S, 151°43'E), 146 m, Coll. W. A. Haswell, 7-VI-1906 (HMCS "Miner"); 5 right and 7 left valves (AMS C 019797 together with *S. strabo*), 12.5 mi E of Cape Byron, New South Wales, Australia (28°38'S, 153°52'E), 203 m, Coll. G. H. Halligan, 10-XI-1902.

Description

Exterior: Shell small (to 12.0 mm), trapezoidal, elongate [H/L = 0.598 ± 0.051 (0.533–0.656)], distinctly inequivalve (left valve slightly larger and more inflated), inflated [W/L = 0.444 ± 0.030 (0.406–0.490)], strongly inequilateral, thick, solid, white under periostracum, with a moderate byssal gape. Sculpture of slightly imbricated commarginal lamellate ridges, made nodulose by closely spaced, wide, high radial riblets. Periostracum thin, pale gray or pale brown, weakly hirsute, with short, thin setae along posteroventral shell margin. Umbones small, low, rounded, well anterior to midline [A/L = 0.329 ± 0.034 (0.290–0.373)]. Interumbonal distance large [L1/L = 0.103 ± 0.014 (0.095–0.130)]. Cardinal area lanceolate, flat, wide, widest in front of umbones, anteriorly and posteriorly demarcated by ridges. Anterior shell end reduced, pointed. Posterior end expanded, drawn out, sharply rounded, slightly flexed to right, with a faint radial carina from beaks to ventral limit of posterior end. Central part of valves with a distinct, deep byssal flexure (more expressed in left valve) from beaks to ventral byssal gape. Anterodorsal margin straight, forming a sharp angle at transition to anterior margin. Anterior margin slightly convex, directed posteriorly, smoothly transitioning to ventral margin. Posterodorsal margin straight, forming a distinct obtuse angle at transition to posterior margin. Posterior margin concave, directed posteriorly, forming a rounded angle at transition to ventral margin. Ventral margin deeply concave in area of byssal gape (concavity more conspicuous in left valve). Ligament opisthodetic, wide, short. Prodissoconch small (length 200–220 μm), D-shaped, smooth.

TABLE 2. *Bentharca asperula*. Summary statistics of shell measurements (mm) and indices for separated valves and whole shells: L – valve length; H – height; W – width; A – anterior end length; L1 – interumbonal distance. Numerator indicates the summary statistics for the right valve, denominator – for the left valve.

	Separated valves							Whole shells					Number of teeth	
Statistics	L	H	W	L	H/L	W/L	A/L	L1	H/L	W/L	A/L	L1/L	Anterior	Posterior
Mean	9.19	6.07	1.98	2.82	0.661	0.215	0.306							
	8.56	5.76	2.03	2.59	0.679	0.241	0.302	0.96	0.666	0.379	0.282	0.111	-	-
SE	0.36	0.25	0.11	0.13	0.011	0.008	0.007							
	0.43	0.25	0.10	0.14	0.013	0.009	0.006	0.06	0.006	0.007	0.009	0.006	-	-
SD	1.29	0.91	0.39	0.45	0.041	0.029	0.025							
	1.76	1.02	0.40	0.59	0.052	0.037	0.024	0.29	0.019	0.025	0.028	0.021	-	-
Min	5.7	3.9	1.2	1.7	0.581	0.172	0.252						4	4
	5.9	4.0	1.4	1.7	0.588	0.178	0.252	0.2	0.643	0.348	0.245	0.072	4	4
Max	10.9	7.3	2.6	3.6	0.726	0.274	0.333						10	8
	11.2	7.3	2.7	3.6	0.800	0.308	0.333	1.5	0.693	0.424	0.326	0.158	10	8
N	13	13	13	13	13	13	13						13	13
	17	17	17	17	17	17	17	23	11	11	11	23	17	17

Interior: Hinge plate slightly curved, narrow. Teeth divided into anterior and posterior sets by a narrow edentulous gap posterior to beaks. Anterior set long, with 7–14 teeth: posteriormost teeth very small, subvertical; anteriormost teeth large, more oblique. Posterior set with 6–9 oblique teeth diverging in varying degree: anteriormost teeth small, lamellate, subhorizontal; posteriormost teeth large, oblique. Adductor scars rounded-triangular, slightly raised, unequal; posterior adductor scar larger than anterior. Shell interior white, with weak radial lines more conspicuous near pallial line.

Variability

Shell shape and proportions vary slightly (Tables 4–5). The shape of the posterior shell end varies from fairly rounded to elongate, angular-rounded. Some specimens have a straight instead of concave posterior shell margin. Relative height and convexity of the shell and the position of beaks are also slightly variable. Teeth of the anterior set vary a great deal in shape. In two specimens, the teeth are conically shaped and irregularly arranged. In another specimen, almost all anterior teeth are curved and expanded dorsally. Anterior and posterior teeth diverge to a markedly variable degree. The canting angle of the anterior teeth varies from 45° to 85°. The canting angle of the posterior tooth set is 10° to 45° for the anteriormost teeth and 30° to 60° for the posteriormost teeth.

Distribution and Habitat

Asperarca xenophoricola occurs off Japan from Suruga Bay, Honshu to Kyushu (Kuroda, 1930; Habe, 1958, 1977, 1981; Habe et al., 1986; Higo et al., 1999; Okutani, 2000), in the South China Sea (Higo et al., 1999), off China (Li, 1984), and off southeastern Australia [33°42'S, 151°43'E, 146 m (AMS C 433165); 28°38'S, 153°52'E, 203 m (AMS C 019797)]. This species has been recorded at depths from 50 m to 500 m, on rubble, attached to shells of gastropods *Xenophora japonica* (Kuroda & Habe, 1971) and *Xenophora pallidula* (Reeve, 1842) (Higo et al., 1999; Okutani, 2000).

Comparisons

This species is easily distinguished from other species of the genus by its less keeled, strongly inequivalve shell with sharply rounded posterior end, deep byssal flexure, and deeply concave posterior and ventral margins. It further differs in that the anterior tooth set is long, with a larger number of teeth and the posterior set consists of small, lamellate, subhorizontal teeth as well as large, oblique teeth.

TABLE 3. *Asperarca nodulosa*. Summary statistics of shell measurements (mm) and indices for separated valves and whole shells: L – valve length; H – height; W – width; A – anterior end length; L1 – interumbonal distance. Numerator indicates the summary statistics for the right valve, denominator – for the left valve.

	Separated valves							Whole shells					Number of teeth	
Statistics	L	H	W	A	H/L	W/L	A/L	L1	H/L	W/L	A/L	L1/L	Anterior	Posterior
Mean	8.72	5.32	2.24	2.62	0.613	0.256	0.300							
	8.90	5.34	2.20	2.62	0.602	0.246	0.294	0.52	0.571	0.468	0.287	0.062	-	-
SE	0.52	0.28	0.21	0.18	0.018	0.011	0.009							
	0.50	0.26	0.17	0.18	0.017	0.007	0.009	0.09	0.014	0.032	0.006	0.013	-	-
SD	1.17	0.62	0.46	0.40	0.040	0.024	0.020							
	1.12	0.59	0.32	0.40	0.037	0.015	0.020	0.29	0.031	0.072	0.013	0.028	-	-
Min	7.0	4.7	1.8	2.1	0.568	0.239	0.277						4	10
	7.3	4.7	1.8	2.1	0.547	0.235	0.274	0.2	0.547	0.393	0.268	0.034	4	10
Max	10.1	6.3	3.0	3.1	0.671	0.297	0.330						6	12
	10.3	6.3	2.8	2.8	0.644	0.272	0.326	1.1	0.625	0.556	0.299	0.107	6	12
N	5	5	5	5	5	5	5						5	5
	5	5	5	5	5	5	5	10	5	5	5	10	5	5

TABLE 4. *Asperarca xenophoricola.* Shell measurements (mm), indices and summary statistics of all characters for separated valves: L – valve length; H – height; W – width; A – anterior end length; L1 – interumbonal distance. Numerator indicates shell measurements and indices for the right valve, denominator – for the left valve.

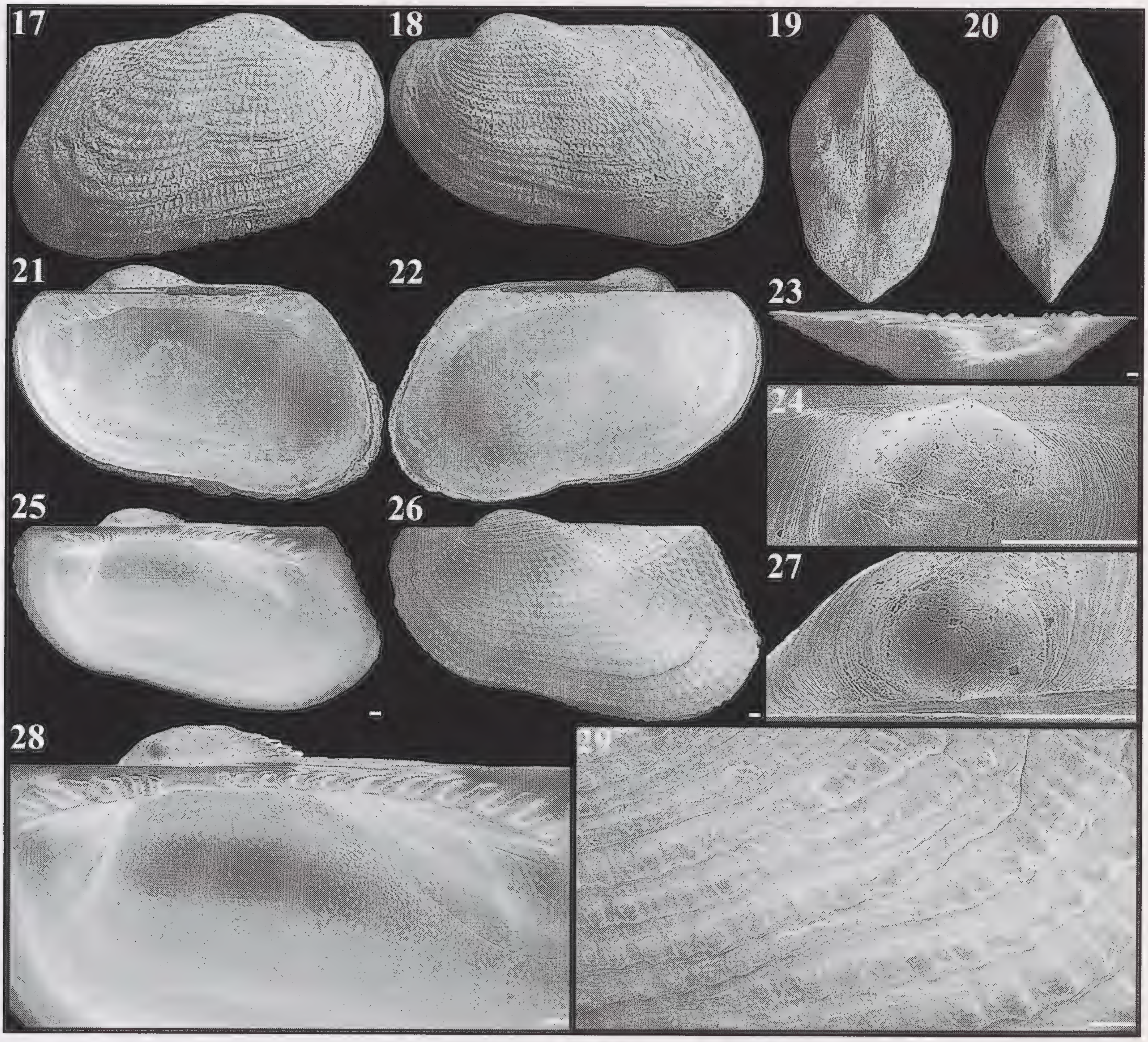
Specimen	L	H	W	A	H/L	W/L	A/L	Number of teeth	
								Anterior	Posterior
NMW 1955.158.10257	9.0	5.3	2.2	3.0	0.589	0.244	0.333	7	7
	9.6	5.7	2.5	3.1	0.594	0.260	0.323	8	8
NMW 1955.158.10256	10.9	5.8	2.3	3.5	0.532	0.211	0.321	14	9
	12.0	6.4	3.1	3.9	0.533	0.258	0.325	14	9
NMW 1955.158.10256	10.0	6.0	2.6	2.9	0.600	0.260	0.290	11	8
	11.0	6.7	3.3	3.3	0.609	0.300	0.300	12	8
NMW 1955.158.10256	5.9	3.7	1.4	2.2	0.627	0.237	0.373	7	7
	6.1	4.0	1.6	2.2	0.656	0.262	0.361	7	6

Statistics	L	H	W	A	H/L	W/L	A/L	Number of teeth	
								Anterior	Posterior
Mean	8.95	5.20	2.13	2.90	0.587	0.238	0.329		
	9.68	5.70	2.63	3.13	0.598	0.270	0.327	-	-
SE	1.09	0.52	0.26	0.27	0.020	0.010	0.017		
	1.29	0.60	0.38	0.35	0.025	0.010	0.013	-	-
SD	2.18	1.04	0.51	0.54	0.040	0.020	0.034		
	2.58	1.21	0.76	0.70	0.051	0.020	0.025	-	-
Min	5.9	3.7	1.4	2.2	0.532	0.211	0.290	7	7
	6.1	4.0	1.6	2.2	0.533	0.258	0.300	7	6
Max	10.9	6.0	2.6	3.5	0.627	0.260	0.373	14	9
	12.0	6.7	3.3	3.9	0.656	0.300	0.361	14	9
N	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4

TABLE 5. *Asperarca xenophoricola.* Shell measurements (mm), indices and summary statistics of all characters for whole shells: L – shell length; H – height; W – width; A – anterior end length; L1 – interumbonal distance.

Specimen	L	H	W	A	L1	H/L	W/L	A/L	L1/L
NMW 1955.158.10257	10.0	5.5	4.9	3.2	1.3	0.550	0.490	0.320	0.130
NMW 1955.158.10257	9.6	5.7	3.9	3.2	1.0	0.594	0.406	0.333	0.104
NMW 1955.158.10256	9.5	5.5	4.0	3.1	0.9	0.579	0.421	0.326	0.095
NMW 1955.158.10256	9.3	5.5	4.3	2.8	0.9	0.591	0.462	0.301	0.097
NMW 1955.158.10256	8.2	5.2	3.6	2.7	0.8	0.634	0.439	0.329	0.098
NMW 1955.158.10256	7.4	5.0	3.3	2.7	0.7	0.676	0.446	0.365	0.095

Statistics	L	H	W	A	L1	H/L	W/L	A/L	L1/L
Mean	9.00	5.40	4.00	2.95	0.93	0.604	0.444	0.329	0.103
SE	0.40	0.10	0.23	0.10	0.08	0.018	0.012	0.009	0.006
SD	0.99	0.25	0.56	0.24	0.21	0.044	0.030	0.021	0.014
Min	7.4	5.0	3.3	2.7	0.7	0.550	0.406	0.301	0.095
Max	10.0	5.7	4.9	3.2	1.3	0.676	0.490	0.365	0.130
N	6	6	6	6	6	6	6	6	6



FIGS. 17–29. *Asperarca nodulosa.* FIGS. 17–18, 21–22: Exterior and interior views of right (length 10.1 mm) and left (length 10.3 mm) valves, Norway, northeastern Atlantic (NMW 1955.158.10253); FIG. 19: Dorsal view of both valves (shell length 11.7 mm), Bergen, Norway, northeastern Atlantic (NMW 1955.158.10252); FIG. 20: Dorsal view of both valves (shell length 9.5 mm (NMW 1955.158.10253); FIGS. 23–25, 27–28: Dorsal and interior views, prodissoconch, and hinge of right valve (NMW 1955.158.10253); FIGS. 26, 29: Exterior view and sculpture of left valve without periostracum (NMW 1955.158.10253). Scale bars = 300 µm.

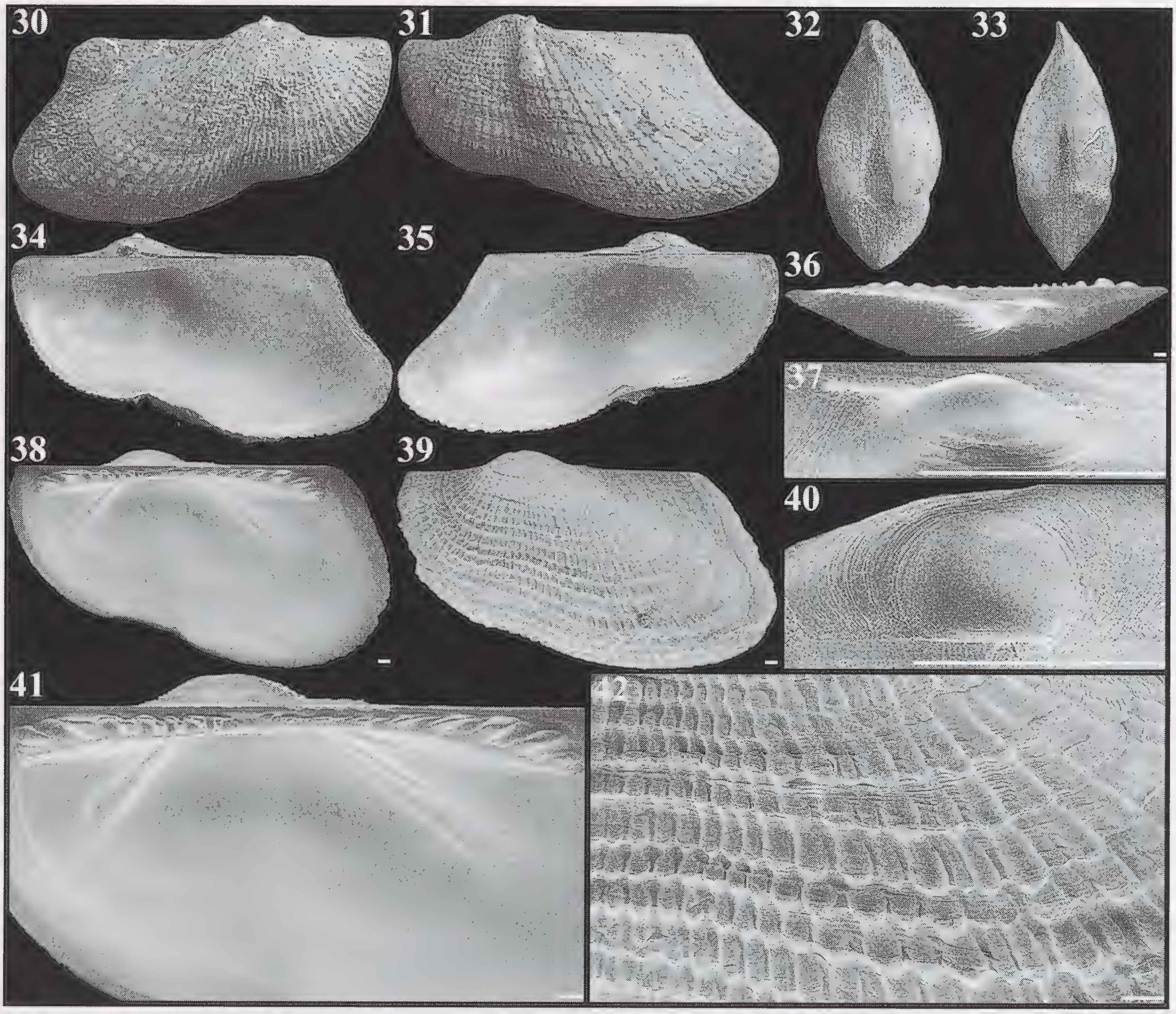
Genus *Deltaodon* Barnard, 1962

Type species

Deltaodon tugelae Barnard, 1962, by original designation.

Diagnosis

Shell small (< 26 mm), thick, moderately inflated, ovate-trapezoidal to trapezoidal, equivalve or slightly inequivalve (left valve sometimes larger and more inflated), strongly inequilateral. Umbones strongly anterior, prosogyrate. Cardinal area very narrow. Sculpture of lamellate commarginal ridges and nodulose, widely spaced radial riblets. Periostracum thin, sometimes slightly hirsute, with short setae along posteroventral margin. Hinge plate broad, curved, most concave and thin posterior to beaks. Teeth divided into anterior and posterior sets by a shallow oblique groove; anterior teeth long, angular, forming an oval group; posterior teeth oblique to subhorizontal. Adductor scars slightly raised. Byssal sinus faint or absent. Ligament external, opisthodetic.



FIGS. 30–42. *Asperarca xenophoricola.* FIGS. 30–31, 34–35: Exterior and interior views of right (length 10.9 mm) and left (length 12.0 mm) valves. Kiji, Japan, northwestern Pacific (NMW 1955.158.10256); FIG. 32: Dorsal view of both valves (shell length 10.0 mm), Kiji, Japan, northwestern Pacific (NMW 1955.158.10257); FIG. 33: Dorsal view of both valves (shell length 9.6 mm (NMW 1955.158.10257); FIGS. 36, 38, 41: Dorsal and interior views and hinge of right valve (NMW 1955.158.10257); FIGS. 37, 40: Prodissoconch of right valve (NMW 1955.158.10257); FIGS. 39, 42: Exterior view and sculpture of left valve without periostracum (NMW 1955.158.10257). Scale bars = 300 μm.

Remarks

This genus was omitted by Newell (1969) and is currently represented by a single species *D. tugelae*. Kilburn (1983) considered *Deltaodon* to be a subgenus of *Samacar* Iredale, 1936, because the posterior teeth of the type species, *D. tugelae*, are subparallel to the dorsal shell margin, as in the holotype of *S. strabo* (Hedley, 1915). However, the present study of type and extensive additional material of *S. strabo* showed that *Deltaodon* is unlike *Samacar* in having a very high shell with a non-hirsute periostracum, and a very broad, strongly curved hinge plate. It further differs in that the hinge teeth are separated into anterior and posterior sets by a very narrow, oblique groove, and very long, angular anterior teeth, sometimes bifid at their lower ends (Barnard, 1962; Kilburn, 1983; Kamenev, 2007). Taking into account the marked differences in shell and hinge morphology, *Deltaodon* is considered as a distinct genus (Kamenev, in press).

Examination of the shell morphologies of *B. rubrotincta* and the type species of the genus *Bentharca* has shown that *B. rubrotincta* differs from *B. asperula* (Figs. 2–16, 43–56, Tables 1–2, 7). The distinctive features of *B.*

TABLE 6. *Dealtaodon tugelae.* Shell measurements (mm), indices and summary statistics of all characters for separated valves: L – valve length; H – height; W – width (after Barnard, 1962).

Specimen	L	H	W	H/L	W/L
SAM Reg. No. A9477	9.0	7.0	2.0	0.778	0.222
SAM Reg. No. A9477	20.0	16.0	4.0	0.800	0.200
SAM Reg. No. A9477	20.0	14.5	3.5	0.725	0.175
SAM Reg. No. A9477	22.0	17.0	5.5	0.773	0.250
SAM Reg. No. A9477	25.0	19.0	5.0	0.760	0.200
Statistics	**L**	**H**	**W**	**H/L**	**W/L**
Mean	19.2	14.7	4.0	0.764	0.206
SE	2.71	2.06	0.61	0.016	0.016
SD	6.06	4.60	1.37	0.031	0.031
Min	9.0	7.0	2.0	0.725	0.175
Max	25.0	19.0	5.5	0.800	0.250
N	5	5	5	5	5

rubrotincta are a thicker shell, with a very narrow cardinal area; stronger radial sculpture; weakly developed hirsute periostracum; broad, strongly curved hinge plate; the presence of oblique groove between the anterior and posterior tooth sets; large, curved anterior teeth; and a very long posterior tooth set with a larger number of teeth. In shell morphology and hinge structure, *B. rubrotincta* is most similar to the type species *Deltaodon* (Figs. 57–61, Table 6). Both have a thick shell, with a very narrow cardinal area, broad and strongly curved hinge plate, with an oblique groove separating the anterior and posterior tooth sets, and large, curved anterior teeth forming an oval group (Tables 1, 6–7). Therefore, *B. rubrotincta* is referred to *Deltaodon.*

Deltaodon rubrotincta (Kuroda & Habe in Habe, 1958)
(Figs. 43–56; Table 7)

Bentharca rubrotincta Kuroda & Habe in Habe, 1958: 252–253.
Bentharca rubrotincta Kuroda & Habe, 1958 – Habe, 1977: 34; Li, 1984: 157, pl. 2, fig. 10.
Bentharca xenophoricola (Kuroda) – Noda, 1988: pl. 1, figs. 13a–15b.
Porterius pacificus (Nomura & Zinbo) – Noda, 1988: pl. 1, fig. 5a–b.

Type Locality

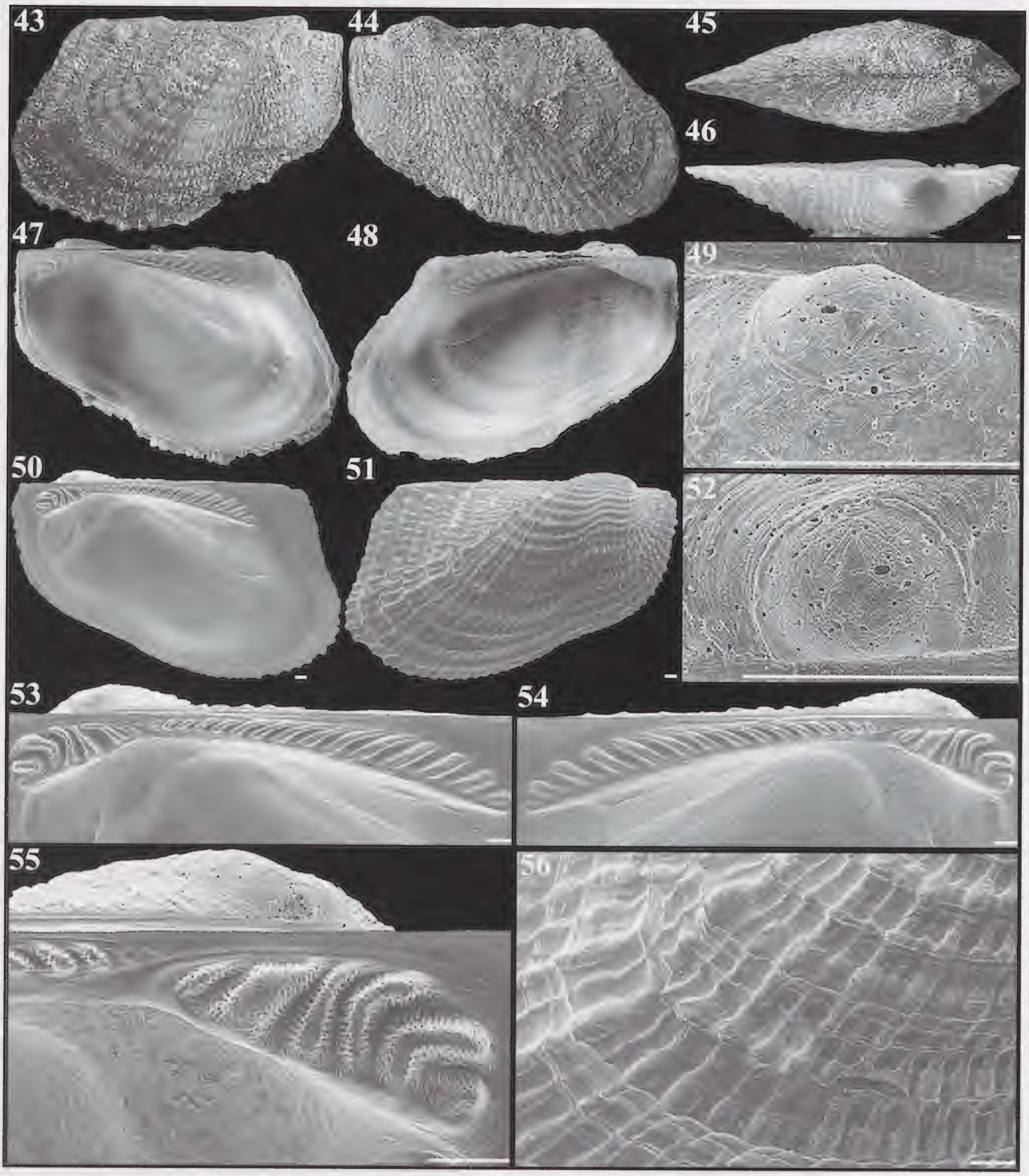
Bungo Strait off western Shikoku, between Kyushu and Shikoku, 110 m, gravel and shells (Habe, 1958; Higo et al., 1999).

Material Examined

Six specimens from Miyako-Jima Islands, Ryuku Archipelago, Japan, 150 m (NSMT 51916).

Description (expanded from Habe, 1958)

Exterior: Shell small (to 12.6 mm), trapezoidal, high [H/L right valve = 0.653 ± 0.024 (0.610–0.691); H/L left valve = 0.667 ± 0.026 (0.632–0.703)], slightly inequivalve (left valve slightly larger and more inflated), moderately inflated [W/L right valve = 0.212 ± 0.013 (0.193–0.232); W/L left valve = 0.230 ± 0.017 (0.206–0.257)], strongly inequilateral, thick, solid, reddish-brown, with white rays and spots. Sculpture of slightly imbricated, wide, commarginal lamellate ridges and high, nodulose, widely spaced radial ribs of varying breadth. Periostracum thin, pale gray, weakly hirsute, with short, thin setae along postero-ventral shell margin. Umbones small, low, rounded, prosogyrate, strongly anterior to midline [A/L right valve = 0.201 ± 0.039 (0.158–0.258); A/L left valve = 0.192 ± 0.035 (0.143–0.242)]. Interumbonal distance very small [L1/L = 0.023 (0.015–0.032)]. Cardinal area needle-shaped in dorsal view, very narrow, widest in front of umbones, span roof-shaped in cross section, anteriorly delineated by termination of sculpture, and posteriorly demarcated by ridges. Anterior shell end reduced, slightly angular. Posterior end expanded, angular rounded, slightly flexed to right, with a faint radial carina from beaks to ventral limit of posterior end. Central part of valves with a distinct byssal flexure (more expressed in left valve), extending from beaks to ventral byssal gape. Anterodorsal margin straight, forming a sharp angle at transition to anterior margin. Anterior margin almost straight, slightly directed posteriorly, smoothly transitioning to ventral margin. Posterodorsal margin straight, forming a distinct obtuse angle at transition to posterior margin. Posterior margin slightly concave, posteriorly directed, forming a rounded angle at transition to ventral



FIGS. 43–56. *Deltaodon rubrotincta*. Miyako-Jima Islands, Ryukyu Archipelago, 150 m (NSMT 51916). FIGS. 43–45, 47–48: Exterior and interior views of right (length 10.9 mm) and left (length 12.0 mm) valves, and dorsal views of both valves; FIGS. 46, 49–50, 52–53: Dorsal and interior views, prodissoconch, and hinge of right valve; FIGS. 51, 56: Exterior view and sculpture of right valve without periostracum; FIGS. 54–55: Hinge of left valve. Scale bars = 300 μm.

margin. Ventral margin slightly concave in area of byssal gape (concavity more conspicuous in left valve). Ligament opisthodetic, narrow, long (more than ½ of posterior part of cardinal area). Prodissoconch small (length 205–215 μm), D-shaped, smooth.

Interior: Hinge plate broad, strongly curved, most concave and thinnest posterior to beaks, strongly widening anteriorly and posteriorly, with a conspicuous ligamental groove under posterior teeth. Teeth divided into anterior and posterior sets by a shallow, oblique groove posterior to beak. Anterior set short, forming an oval group of 4–7 teeth; posterior set long, with 9–18 teeth. Anterior teeth long, large, oblique, mostly curved, boomerang-shaped. Posterior teeth long, increasing in size, oblique, diverging to a varying degree, inner teeth slightly curved. Adductor scars rounded-triangular, slightly raised, unequal; posterior adductor scar larger than anterior. Shell interior reddish-brown, with white rays and spots, and with weak radial lines more conspicuous near pallial line.

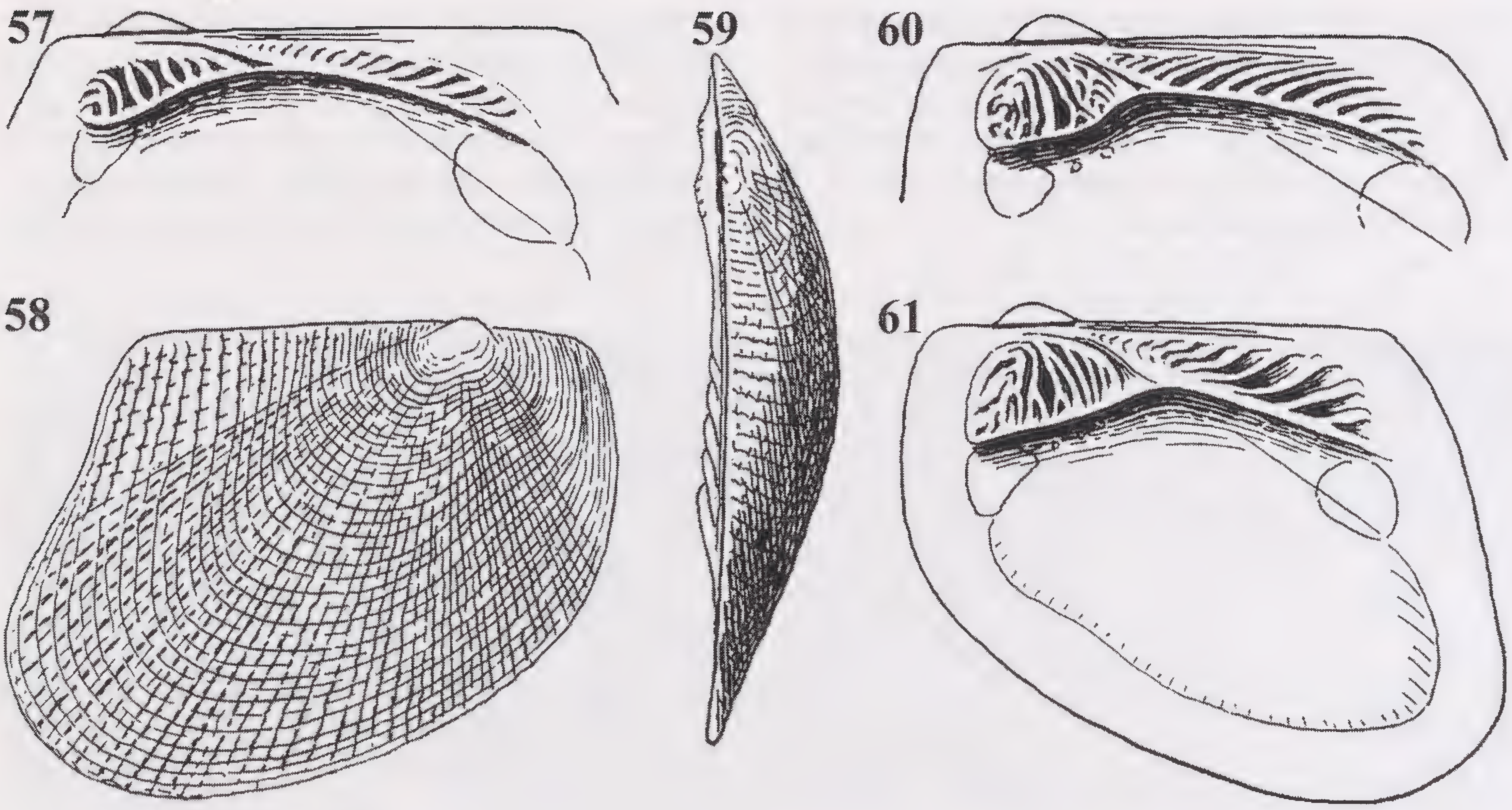
Variability

Shell shape and proportions vary little (Table 7). Relative height and convexity of the shell and the location of the beaks are slightly variable. The number of teeth in the anterior and

TABLE 7. *Deltaodon rubrotincta*. Shell measurements (mm), indices and summary statistics of all characters for separated valves: L – valve length; H – height; W – width; A – anterior end length; L1 – interumbonal distance. Numerator indicates shell measurements and indices for the right valve, denominator – for the left valve. NM – not measured.

Specimen	L	H	W	A	L1	H/L	W/L	A/L	L1/L	Number of teeth	
										Anterior	Posterior
Holotype (after Habe, 1958)	8.2	5.0	1.9	NM		0.610	0.232	NM		NM	NM
	9.5	6.0	2.2	NM	NM	0.632	0.232	NM	NM	NM	NM
NSMT 51916	11.4	7.5	2.2	1.8		0.658	0.193	0.158		7	18
	12.6	8.0	2.6	1.8	0.4	0.635	0.206	0.143	0.032	7	18
NSMT 51916	9.4	6.5	2.0	1.9		0.691	0.213	0.202		5	14
	10.1	7.1	2.6	2.0	0.2	0.703	0.257	0.198	0.020	6	14
NSMT 51916	8.9	5.8	1.8	1.5		0.652	0.202	0.169		6	16
	9.6	6.4	2.1	1.7	0.2	0.667	0.219	0.177	0.021	6	16
NSMT 51916	8.2	5.4	1.7	1.5		0.659	0.207	0.183		4	12
	8.6	5.9	2.0	1.5	0.2	0.686	0.233	0.174	0.023	5	13
NSMT 51916	3.8	2.5	0.8	0.9		0.658	0.211	0.237		4	11
	4.1	2.8	0.9	0.9	0.1	0.683	0.220	0.220	0.024	4	11
NSMT 51916	3.1	2.0	0.7	0.8		0.645	0.226	0.258		4	9
	3.3	2.2	0.8	0.8	0.05	0.667	0.242	0.242	0.015	4	10

Statistics	L	H	W	A	L1	H/L	W/L	A/L	L1/L	Number of teeth	
										Anterior	Posterior
Mean	7.57	4.96	1.59	1.40		0.653	0.212	0.201			
	8.26	5.49	1.88	1.45	0.19	0.667	0.230	0.192	0.023	-	-
SE	1.14	0.76	0.22	0.19		0.009	0.005	0.016			
	1.27	0.82	0.28	0.20	0.05	0.010	0.006	0.014	0.002	-	-
SD	3.02	2.02	0.59	0.46		0.024	0.013	0.039			
	3.36	2.17	0.74	0.49	0.12	0.026	0.017	0.035	0.006	-	-
Min	3.1	2.0	0.7	0.8		0.610	0.193	0.158		4	9
	3.3	2.2	0.8	0.8	0.05	0.632	0.206	0.143	0.015	4	10
Max	11.4	7.5	2.2	1.9		0.691	0.232	0.258		7	18
	12.6	8.0	2.6	2.0	0.4	0.703	0.257	0.242	0.032	7	18
N	7	7	7	6	6	7	7	6	6	6	6
	7	7	7	6	6	7	7	6	6	6	6



FIGS. 57–61. *Deltaodon tugelae.* FIG. 57: Hinge teeth of right valve of juvenile (length 9.0 mm); FIG. 58: Exterior view of right valve (length 20.0 mm); FIG. 59: Dorsal view of right valve. FIGS. 60–61: Hinge teeth of right valves of two shells to show variation (after Barnard, 1962).

posterior sets depends on the size of the specimen. The posterior teeth diverge to a variable degree. The canting angle of posterior teeth varies from 30° to 60°.

Distribution and Habitat

Deltaodon rubrotincta occurs off Japan from the Kii Peninsula to the Yaeyama Islands (Habe, 1958, 1981; Higo et al., 1999), in the East China Sea and South China Sea (Higo et al., 1999), and off China (Li, 1984). This species was recorded at depths from 100 m to 200 m, attached to rocks and stones (Habe, 1958, 1977; Higo et al., 1999).

Comparison

In contrast to *D. tugelae*, *D. rudrotincta* has a more elongate, reddish-brown shell, a narrower hinge plate, and shorter anterior teeth unbifid at their lower ends (Tables 1, 6–7).

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ONCOMELANIA HUPENSIS (GASTROPODA: RISSOOIDEA), INTERMEDIATE HOST OF *SCHISTOSOMA JAPONICUM* IN CHINA: GENETICS AND MOLECULAR PHYLOGENY BASED ON AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS

Yi-Biao Zhou[1,2], Mei-Xia Yang[3], Gen-Ming Zhao[1], Jiang-Guo Wei[1] & Qing-Wu Jiang[1,2]*

ABSTRACT

Schistosomiasis japonica is one of the most serious parasitic diseases in China. Subspecies of the pomatiopsid snail species *Oncomelania hupensis* transmit the human blood fluke *Schistosoma japonicum,* the parasite causing the disease. In at least one study involving the subspecies *Oncomelania hupensis robertsoni*, there was no clear phylogenetic concordance between the mitochondrial and nuclear data, and it was strongly recommended that future studies incorporate more data from nuclear loci in order to better understand phylogeography and host-parasite coevolution (Wilke et al., 2006). In this paper, we explore genetic diversity based AFLP data involving 25 populations throughout mainland China. AFLP is a DNA fingerprinting technique that detects the polymorphism of the whole genome without prior knowledge of the nucleotide sequence, with the high degree of reproducibility and small amount of template DNA needed.

Our AFLP data show that: (1) the genetic diversity within an *O. hupensis* population increases gradually with snail sample size, and when the sample size is more than 30 individuals, the genetic variation within one *O. hupensis* population trends to stabilization. (2) There is high intra-population genetic variation on the mainland of China, and these intra-population genetic variations from different areas differ significantly. (3) The considerable genetic differentiation occurs throughout China. (4) The genetic variation among populations of *O. h. hupensis* is higher than that of *O. h. robertsoni.* (5) The patterns of genetic differentiation are basically consistent with geographical distribution of snail populations. Our results are similar to the results of allozymes and COI gene sequences, excluding a snail population from Guangxi Province (Gx-1), indicating that there are three distinct subspecies in mainland China, namely *O. h. hupensis*, *O. h. robertsoni*, and *O. h. tangi*. However, our results do not support that the snail population from Guangxi Province belongs to *O. h . hupensis*.

Key words: *Schistosomiasis japonicum*, *Ocomelania hupensis*, China, molecular genetics, phylogeny.

INTRODUCTION

Schistosomiasis japonica is one of the most serious parasitic diseases in China, with a documented history of over 2100 years. By the end of 2003, an estimated 843,011 people were infected with *S. japonicum*. Among them, there were 1,114 cases with acute schistosomiasis and 24,441 cases suffering from advanced schistosomiasis. The human population at risk of infection was estimated around 65 million, and there were 74,000 infected cattle. The intermediate snail host, *Oncomelania hupensis,* infested an area of 3,787 km^2 (Zhou et al., 2005a). Basic epidemiological and malacological research were discouraged in China as the theoretical goal of preventing *schistosomiasis japonicum* transmission seemed attainable by introduction of effective programs of chemotherapy (using Praziquantel) to eliminate adult worms in the human or livestock hosts, and proper sanita-

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tion concurrent together with the indiscriminant application of molluscicides to exterminate the snails. Although such integrated programs have had dramatic success in some areas of China (Jiang et al., 2002), the sustained effort required for their maintenance in contiguous water bodies requires considerably more resources than are available in many affected areas (World Health Organization, 1993). Additionally, synthetic molluscicides can cause serious environmental damage to freshwater ecosystems. In China, water development schemes, such as the Three Gorges Dam on the Yangtze River and the south-to-north water transfer project, may encourage growth and spread of *Oncomelania hupensis*, creating a need for further basic malacological research.

The subspecies and populations of *Oncomelania hupensis* differ in shell size and structure, breeding environment, growth rates, population genetics, and potential for infection by *S. japonicum* (Yuan et al., 1984; Hope & McManus, 1994; Davis et al., 1995, 1999a, 2006; Wilke et al., 2000; Zhou et al., 2005b). Based on congruence of allozymes and mitochondrial cytochrome oxidase I [COI] gene sequences (Davis et al., 1995, 1999a, b; Wilke et al., 2000; Shi et al., 2002), there are three distinct clades of *Oncomelania hupensis* in the mainland China, widely separated biogeographically. These have been assigned to three subspecies. *Oncomelania h. hupensis* thrives throughout the Yangtze River drainage system below the Three Gorges to Shanghai, Jiangsu, and Zhejiang provinces (some populations are dispersed at high altitudes). This subspecies has populations with smooth or ribbed shells (Davis et al., 2006). *Oncomelania h. robertsoni* is located in Sichuan and Yunnan provinces at high elevations (500 m, most 1,000 m above sea level) above the Three Gorges of the Yangtze River and has the ancestral (pleisiomorphic) small, smooth shell without a varix (thickening of the outer lip of the shell) (Davis, 1979; Davis et al., 1995); *O. h. tangi* is found in Fujian Province along the southern coast of China, opposite Taiwan, separated from the Yangtze River by high mountain ranges. The shells are smooth but have a doubly thick varix, and their allometry differs from that of the other two subspecies in that their shells are proportionally much wider for their length.

As was shown by previous allozyme studies, the introduction of new molecular-based methodologies has the potential to greatly improve differentiating populations and higher taxa. In recent years, some universal PCR primers were used to amplify targets in nuclear and mitochondrial genomes across a broad range of taxa (Simon et al., 1994; Pedersen, 1996), and the introduction of arbitrary primer mediated DNA fingerprinting (Williams et al., 1990; Welsh & McClelland, 1990; Caetano-Anolles et al., 1991; Vos et al., 1995). The choice of these universal primer methods for species identification and elucidation of relationships in tropical freshwater snails was discussed by Jones et al. (1999). The Randomly Amplified Polymorphic DNAs (RAPDs) method was successfully utilized for species identification and clarification of genetic relationships among planorbid snails in Central and East Africa (Jones et al., 1999). In contrast, Amplified Fragment Length polymorphisms (AFLPs) combine RAPDs and RFLPs to assay anonymous sequences over the entire genome, but with the major advantage of being more robust and reliable than other arbitrary primer-mediated DNA fingerprinting methods, because amplification is performed under more stringent reaction conditions (Vos et al., 1995). The AFLP markers have been used to infer phylogenetic relationships of closely related species based on measures of genetic distance (Heun et al., 1997; Semblat et al., 1998). Based on analyses mtDNA, LSU rRNA, and AFLPs of *O. hupensis robertsoni* specimens, Wilke et al. (2006) found that there was no clear phylogenetic concordance between the mitochondrial and nuclear data in *Oncomelania hupensis robertsoni* (in contrast with *O. h. hupensis*). They strongly recommended that future studies incorporate more data from nuclear loci in order to better understand phylogeography, population genetics, and host-parasite coevolution.

The purposes of this paper are (1) to use AFLP methodology to assess the degree to which AFLPs derived relationships are congruent with the allozymes and COI results; (2) to determine the degree to which AFLPs data enables analysis of population structure.

MATERIALS AND METHODS

Locality Data

Oncomelania is distributed in twelve provinces of mainland China. We collected samples of 25 snail populations from ten provinces. We did not find sufficient samples for studying in Shanghai and Guangdong prov-

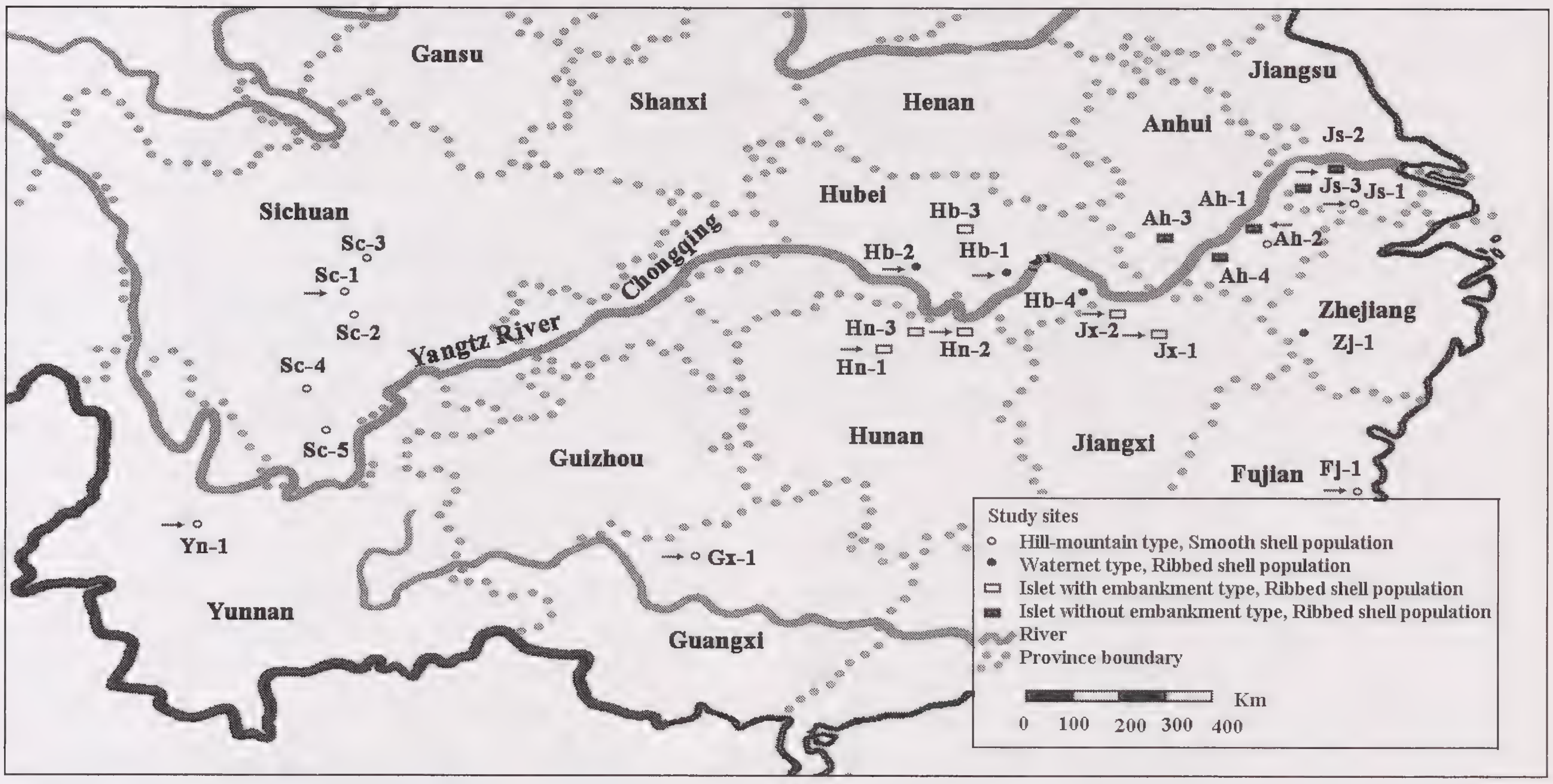


FIG. 1. Localities studied. Populations pointed out by arrows were used for studies of intra-population variation.

inces. A total of 760 uninfected individuals from 25 *O. hupensis* populations were studied (Fig.1). Analyses were done on 40 individuals randomly sampled from the *O. hupensis* population from Hn-2; 30 individuals were analyzed from each of the other populations. Locality information is given in Table 1.

Snail Selection

Individuals (120) were collected at random from each *O. hupensis* population. The snails were isolated without food, in an antibiotic solution (ampicillin 100 µg/ml) and left overnight at 4°C to minimize prokaryotic growth prior to DNA extraction. Then the cercaria shedding test was used to examine whether they were infected with *S. japonicum*. Those positive snail individuals were eliminated. The remaining individuals were crushed and examined microscopically for larval trematodes, then the negative individuals were selected for DNA isolation.

DNA Extraction

Genomic DNA was extracted from the foot of each snail individual using a modified CTAB protocol (Enjalbert et al., 2002). The DNA concentrations were determined by O.D. measurement using a BioPhotometer (Eppendorf, Hamburg, Germany). All samples were then diluted to a concentration of 50ng/µl. After dilution, additional measurements were performed to ensure the correct concentration.

Population Used to Study Variation with Increased Sample Size and Number of AFLP Molecular Loci

The Hn-2 population was selected to analyze how much variation increasing with sample size and the number of AFLP molecular loci.

Populations Used to Study Intra-Population Genetic Variation

Thirteen snail populations (Yn-1, Js-1, Sc-1, Fj-1, Gx-1, Js-2, Ah-1, Jx-1, Jx-2, Hn-1, Hn-2, Hb-1, Hb-2) were selected from the 25 snail populations to determine the amount of intra-population variation. Of these snail populations, ten populations (Hn-1, Hn-2, Js-1, Js-2, Gx-1, Hb-1, Hb-2, Ah-1, Jx-1 and Jx-2) from six provinces belong to *O. h. hupensis*; two populations (Yn-1 and Sc-1) are *O. h. robertsoni*; one population (Fj-1) is *O. h. tangi*.

DNA Pool Construction

For each snail population, 30 µl from 30 snail individuals (1 µl per individual) from the same population were pooled together to assess the molecular genetics of *O. hupensis*.

TABLE 1. Locality information for twenty five snail populations of *Oncomelania hupensis* studied.

Locality code	Shell sculpture	Varix	Locality	Latitude	Longitude
Hn-1	Ribbed	Yes	Hunan, Nanxian County, Babaigong Township, Xinshang Village	29.1365°N	112.3329°E
Hn-2	Ribbed	Yes	Hunan,Yueyang City, Junshan District, Liulinzhou Township, Changjiang Village	29.3881°N	112.8595°E
Hn-3	Ribbed	Yes	Hunan, Hua Rong County, Xing Fu Township, Dong Jia Village	29.3060°N	112.7202°E
Yn-1	Smooth	No	Yunnan, Dali City, Yinqiao Township, Wuliqiao Village	25.6686°N	100.1789°E
Js-1	Smooth	Yes	Jiangsu, Yixing City, Hufu Township, Zhanggong Village	31.2254°N	119.8871°E
Js-2	Ribbed	Yes	Jiangsu, Dantu County, Shiye Township, Xintan Village	32.2107°N	119.2855°E
Js-3	Ribbed	Yes	Jiangsu, Jiang Ning District, Tong Jing Township, Xin Ji Zhou Village	31.7932°N	118.5447°E
Sc-1	Smooth	No	Sichuan, Pujiang County, Changqiu Township, Sanhe Village	30.2167°N	103.6370°E
Sc-2	Smooth	No	Sichuan, Dan Leng County, Yang Chang Township, Xu Ba Village	29.9131°N	103.5502°E
Sc-3	Smooth	No	Sichuan, Guang Han City, Shuang Quan Township, Long Quan Village	30.9952°N	104.4267°E
Sc-4	Smooth	No	Sichuan, Xi Chang City, Chuan Xing Township, Xin Long Village	27.8670°N	102.3237°E
Sc-5	Smooth	No	Sichuan, Pu Ge County, Te Zi Township, Chang Shou Village	27.6129°N	102.6653°E
Fj-1	Smooth	Yes	Fujian, Fuqing City, Donghan Township, Donghan Village	25.4130°N	119.6140°E
Gx-1	Smooth	Yes	Guangxi, Yizhou City, Fulong Township, Jingkou Village	24.5009°N	108.1668°E
Hb-1	Ribbed	Yes	Hubei, Wuhan City, Caidian District, Xiaosi Township, Wagou Village	30.4471°N	113.6889°E
Hb-2	Ribbed	Yes	Hubei, Jiangling County, Zishi Township, Fanyuan Village	30.1842°N	112.4084°E
Hb-3	Ribbed	Yes	Hubei, Han Chuan City, Liu Ge Township, Xi Si Tai Village	30.7575°N	113.8114°E
Hb-4	Ribbed	Yes	Hubei, Yang Xin County, Mu Gang Township, Xin Hu Village	29.7265°N	115.2476°E
Ah-1	Ribbed	Yes	Anhui, Tongling County, Laozhou Township, Guanghui Village	30.9723°N	117.7477°E
Ah-2	Smooth	Yes	Anhui, Tong Ling County, Shun An Township, Chang Shan Village	30.9538°N	117.9407°E
Ah-3	Ribbed	Yes	Anhui, Cong Yang County, Feng Yi Township, Feng Yi Village	30.7106°N	117.4407°E
Ah-4	Ribbed	Yes	Anhui, Gui Chi District, Mei Long Township, Guo Gang Village	30.7673°N	117.6423°E
Jx-1	Ribbed	Yes	Jiangxi, Douchang County, Zhouxi Township, Chaipeng Village	29.1822°N	116.3912°E
Jx-2	Ribbed	Yes	Jiangxi, Xingzi County, Xinchi Township, Zhuxi Village	29.3098°N	116.0047°E
Zj-1	Ribbed	Yes	Zhejiang, Jiangshan County, Wangyao Township, Dahe Village	28.6890°N	118.6597°E

Amplified Fragment Length Polymorphism (AFLP) Reaction

AFLP protocol was adapted from Mikkonen et al. (2005) with some modifications as follows: two hundred nanograms of genomic DNA was digested with 8 units of each restriction Enzyme *PstI* and *MseI* (New England Biolabs), and then restriction fragments were ligated to the *PstI* adapter and *MseI* adapter at 37°C for 5 h in a restriction-ligation buffer [2.5μl 10 × Reaction buffer, 1 μl *PstI* / *MseI* adapter, 2 μl *PstI* / *MseI*, 2.5 μl 10mM ATP, 3 unit of T4 DNA Ligase, 7 μl Ultrapure Water]. The final volume of the mixture was 20 μl. After a 1 : 20 dilution in ultrapure water, ligation products were used directly (no streptavidine purification) for the pre-amplification, using *Pst* and *Mse* primers (2 ng/μL) with no selective nucleotides.

In the pre-amplification, 2 μl of ligated restriction fragments was used as template. 1 μl unlabelled *PstI* -0 primer and *MseI* -0 primer (MWG-Biotech AG) were added to a amplification buffer [1 μl dNTPs, 2.5 μl 10Reaction buffer, 1 unit of T4 DNA Ligase, 18 μl Ultrapure Water], giving a total volume of 25 μl. Preselective amplification was performed at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 80 s and at last 72°C for 5 min.

In the selective amplification, the products of the pre-amplification were diluted 1 : 20 in ultrapure water, and followed by a specific amplification with three selective nucleotides on both *MseI* and *PstI* primers (Table 2). Selective DNA amplification was performed in a 20 μl reaction mixture consisting of 2 μl of template DNA from the preselective PCR, 2 μl 10x Reaction buffer, 1 μl dNTP, 1 μl fluorescence labeled *PstI* primer (5 ng/μl), 1 μl fluorescence labeled *MseI* primer (30 ng/μl), 1 unit of T4 DNA Ligase, and 12.5 μl Ultrapure Water. Selective amplification was done by incubating at 94°C for 2 min, then one cycle of 94°C for 30 s, 65°C for 30 s and 72°C for 80 s. The annealing temperature was then reduced by 0.7°C in each subsequent cycle until attaining 56.6°C (12 cycles), followed by an extra 23 cycles at a 94°C for 30 s, 55°C for 30, 72°C for 80 s, and at last 72°C for 5 min.

PCR products were separated electrophoretically using a 6% polyacrylamide gel and an automatic ABI 377 DNA Sequencer (GMI, Inc.). The resulting band patterns were analyzed visually. AFLP fragments of strong intensity were scored as binary characters for each isolate.

Data Analysis

The AFLP markers, treated as alleles, were analyzed following the approach used for RAPD (Random Amplified Polymorphic DNA) markers (Luch, 1990; Vekemans et al., 2002). The percentage of polymorphic loci, Nei's gene diversity (Nei, 1973), and Shannon's information index (Lewontin et al., 1972) were computed using the software package PopGen32 (version 1.32) (Yeh, 1999). Pairwise similarities (GS_{Dice}) were calculated between snail populations using Dice's coefficient (Nei & Li, 1979) by Simqual program of the software packages NTSYpc (Rohlf, 1998), and the similarity coefficients (GS_{Dice}) were converted to genetic distance (Genetic distance D = 1-GS_{Dice}).The

TABLE 2. 12 primer combinations used for AFLP analysis, and their sequences.

Primer Pair	*PstI*	Primer Sequence	*MseI*	Primer Sequence
Pre-	P0	5'-GACTGCGTACATGCA-3'	M0	5'-GATGAGTCCTGAGTAA-3'
A7	P1	5'-GACTGCGTACATGCAGAA-3'	M7	5'-GATGAGTCCTGAGTAACTG-3'
A8	P1	5'-GACTGCGTACATGCAGAA-3'	M8	5'-GATGAGTCCTGAGTAACTT-3'
B4	P2	5'-GACTGCGTACATGCAGAC-3'	M4	5'-GATGAGTCCTGAGTAACAT-3'
B6	P2	5'-GACTGCGTACATGCAGAC-3'	M6	5'-GATGAGTCCTGAGTAACTC-3'
B7	P2	5'-GACTGCGTACATGCAGAC-3'	M7	5'-GATGAGTCCTGAGTAACTG-3'
B8	P2	5'-GACTGCGTACATGCAGAC-3'	M8	5'-GATGAGTCCTGAGTAACTT-3'
F5	P6	5'-GACTGCGTACATGCAGTC-3'	M5	5'-GATGAGTCCTGAGTAACTA-3'
F7	P6	5'-GACTGCGTACATGCAGTC-3'	M7	5'-GATGAGTCCTGAGTAACTG-3'
G5	P7	5'-GACTGCGTACATGCAGTG-3'	M5	5'-GATGAGTCCTGAGTAACTA-3'
G7	P7	5'-GACTGCGTACATGCAGTG-3'	M7	5'-GATGAGTCCTGAGTAACTG-3'
G8	P7	5'-GACTGCGTACATGCAGTG-3'	M8	5'-GATGAGTCCTGAGTAACTT-3'
H8	P8	5'-GACTGCGTACATGCAGTT-3'	M8	5'-GATGAGTCCTGAGTAACTT-3'

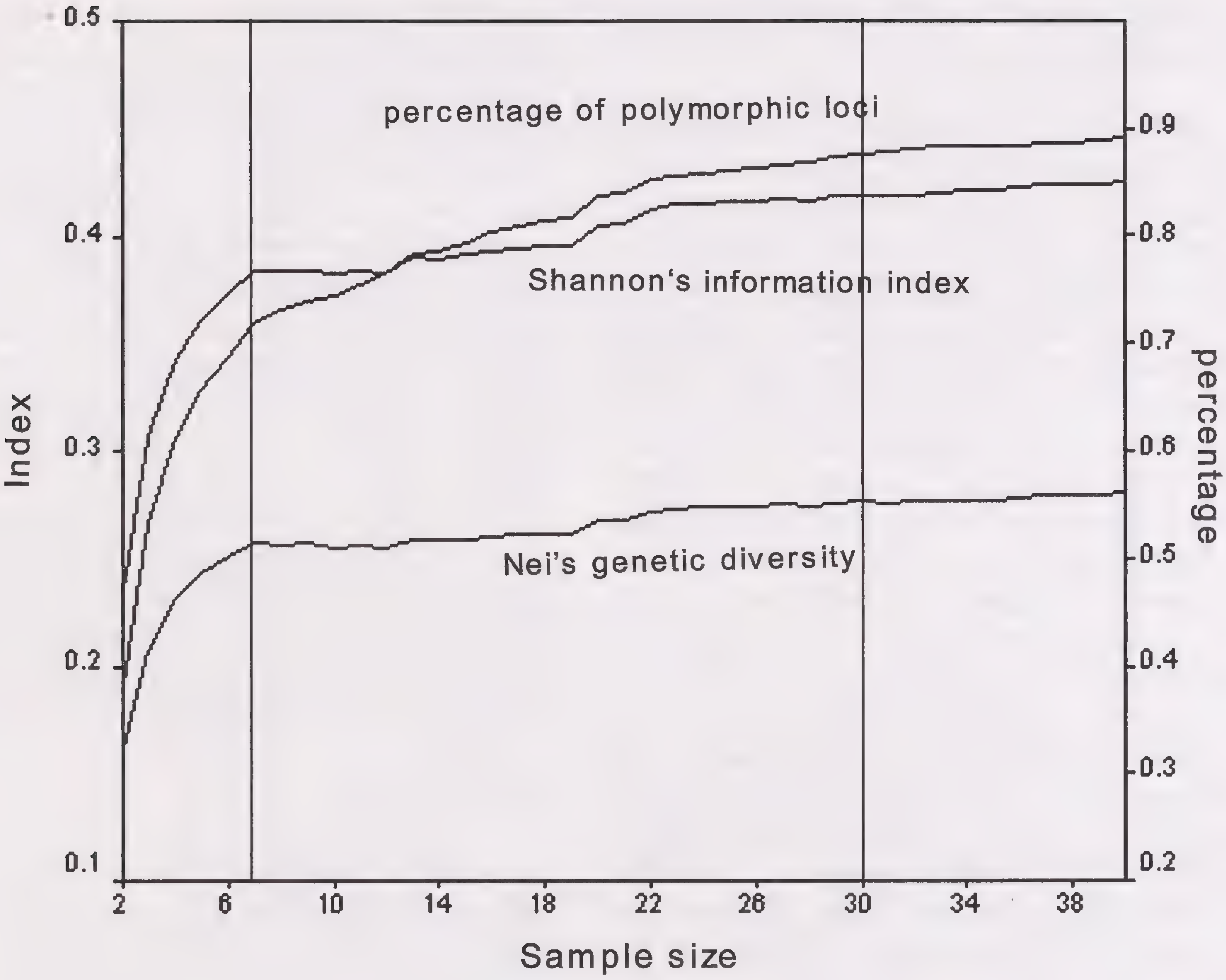


FIG. 2. Information on intra-population genetic diversity with snail sample size increasing.

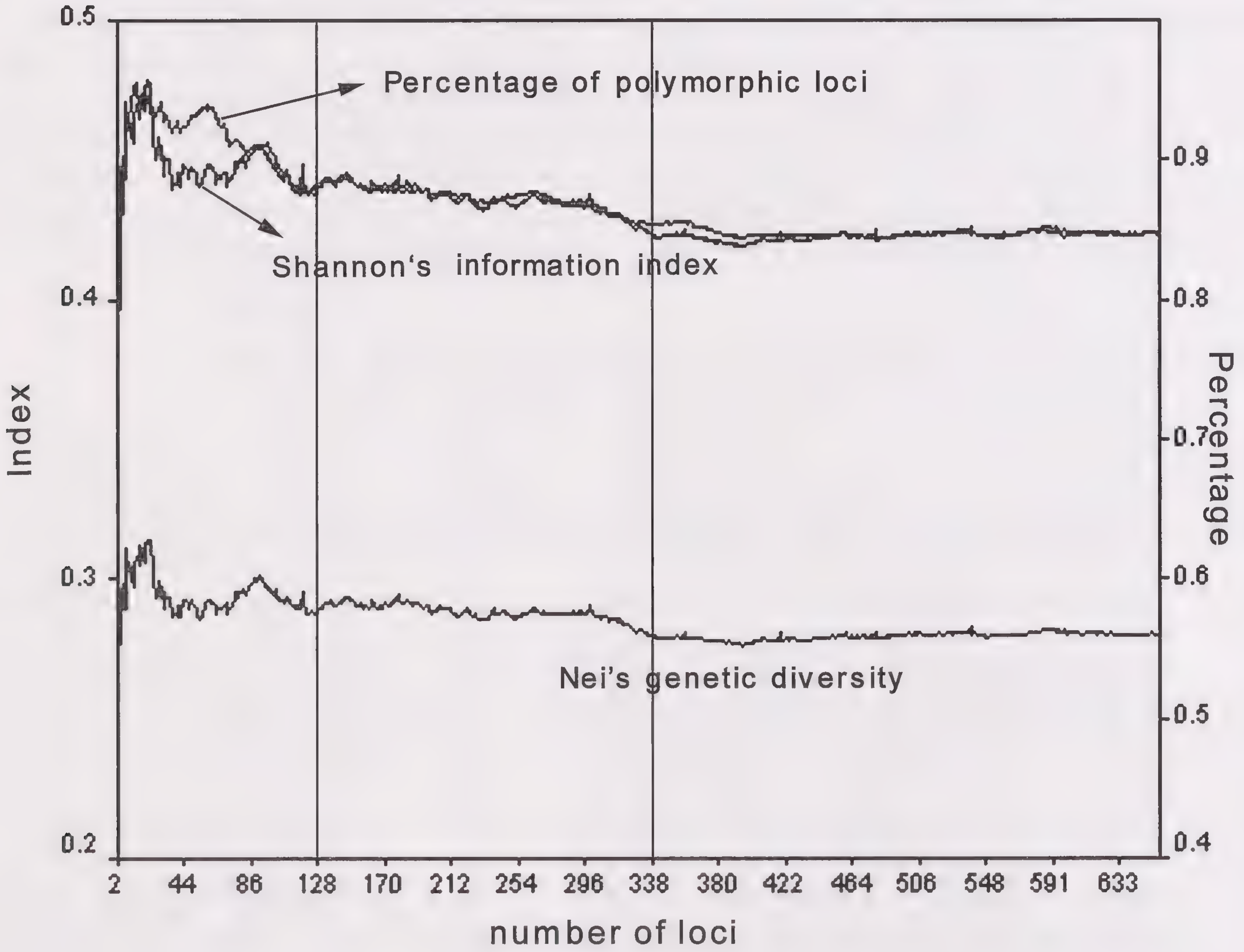


FIG. 3. Information on intra-population genetic diversity with the number of AFLP loci increasing.

genetic diversities were compared among multiple snail populations with Kruskal-Wallis H nonparametric test, between subspecies of *O. hupensis* with Mann-Whitney U of the nonparametric test. GS_{Dice} were compared between subspecies of *O. hupensis* with bootstrap approach. The other data analysis and graphs drawing were performed using the software packages Microsoft Excel (Microsoft Corporation, USA) and SPSS (SPSS Inc., Chicago, USA).

RESULTS

Relationship between Stability of Information on Genetic Variation and Sample Size

The AFLP method was used to analyze the 40 individuals of *O. hupensis* from Hn-2 with 12 primer pairs. 42–74 distinct fragments with a size in the range of 50–490 bp were yielded for each primer combination, and the percentage of polymorphic loci was approximately 82.9%–93.9%. Overall, the percentage of AFLP polymorphic loci, Nei's gene diversity, and Shannon's information index increased gradually with increasing sample size. There were three phases: (a) The rapidly changing phase: when sample size increased from 2 to 7 snail individuals, the three indices above increased rapidly. (b) The slowly changing phase: when sample size increased from 7 to 30 individuals, the values of the three indices increased slowly. (c) The stable phase: when sample size was more than 30 snails, the values of these indices trended to stabilization (Fig. 2). The snail individuals in the order on the x-axis were randomized.

Relationship Between Stability of Information on Genetic Variation and Number of AFLP Molecular Loci

Thirty out of 40 snails from Hn-2 above were selected at random to analyze how much variation increased with the number of AFLP loci. With the increase in the number of AFLP loci, the change of the percentage of polymorphic loci and the indices of genetic diversity was divided approximately into three phases as above: (a) The phase of rapid change: when the number of AFLP loci increased from 2 to 128, the values of the percentage of polymorphic loci and the indices of genetic diversity fluctuated rapidly. (b) The phase of slow change: when it increased from 128 to 338, the values of these indices fluctuated slowly. (c) The stable phase: when the number of AFLP loci was more than 338, the values of these indices trended to stabilization (Fig. 3). The loci in the order on the x-axis were randomized.

Intra-Population Genetic Diversity

Thirteen populations (Fig. 1) were studied by AFLP method with eight primer pairs (namely A7, A8, B4, B6, B7, G5, G7, and G8). Within single locality population, the number of fragments amplified by AFLP method ranged from 403 to 472 with the number of

TABLE 3. Genetic diversities within 13 populations of *Oncomelania hupensis.*

Population code	No. loci	No. of polymorphic loci	Percentage of polymorphic loci (%)	Nei's gene diversity	Shannon's information index
Yn-1	436	379	86.87	0.322 ± 0.0026	0.476 ± 0.0035
Js-1	403	314	77.92	0.270 ± 0.0030	0.404 ± 0.0041
Sc-1	424	314	74.03	0.285 ± 0.0031	0.421 ± 0.0043
Fj-1	430	304	70.61	0.253 ± 0.0029	0.377 ± 0.0042
Gx-1	405	226	55.80	0.191 ± 0.0031	0.287 ± 0.0045
Js-2	467	418	89.51	0.333 ± 0.0023	0.493 ± 0.0031
Ah-1	426	370	86.85	0.322 ± 0.0025	0.478 ± 0.0035
Jx-1	434	378	87.10	0.313 ± 0.0026	0.465 ± 0.0035
Jx-2	472	440	93.22	0.345 ± 0.0022	0.510 ± 0.0029
Hn-1	459	403	87.77	0.339 ± 0.0025	0.500 ± 0.0034
Hn-2	453	391	86.31	0.276 ± 0.0027	0.420 ± 0.0037
Hb-1	433	371	85.88	0.330 ± 0.0026	0.485 ± 0.0035
Hb-2	415	343	82.55	0.302 ± 0.0029	0.450 ± 0.0040

polymorphic loci from 226 to 440; a percentage of polymorphic loci ranged from 55.80% to 93.22%, and the Nei's gene diversity from 0.191 to 0.345; the Shannon's information index ranged from 0.287 to 0.510. The genetic diversities within *Oncomelania* populations from different areas differed significantly (Nei's gene diversity $\chi^2 = 2500.56$, $P < 0.01$; Shannon's information index $\chi^2 = 2501.20$, $P < 0.01$). Of thirteen snail populations, the genetic diversity within Gx-1 was the lowest, and the percentage of polymorphic loci, Nei's gene diversity, Shannon's information index and average coefficient of similarity were 55.80%, 0.191, 0.287 and 0.904 respectively; whereas the percentage of polymorphic loci, Nei's gene diversity and Shannon's information index of Jx-2 were the most and were 93.22%, 0.345 and 0.510 respectively (Table 3).

Inter-Population Genetic Variation

DNA pools of 25 five snail populations were amplified using the AFLP method with eight primer pairs (A7, A8, B4, B6, B7, G5, G7, and G8). These distinct amplified fragments with a size in the range of 50 to 325 bp were selected to analyze the genetic variation among snail populations.

Among 25 snail populations, Nei's gene diversity and Shannon's information index were 0.280 and 0.426 respectively; genetic distance D from 0.169 to 0.306, and Nei's unbiased genetic distance from 0.225 to 0.452. Among six snail populations of *O. h. robertsoni*, genetic distance D was from 0.170 to 0.235, and Nei's unbiased genetic distance was from 0.225 to 0.334. Among 18 snail populations of *O. h. hupensis*, genetic distance D was in the range of 0.176 to 0.289, and Nei's unbiased genetic distance was in the range of 0.229 to 0.413. Whether Nei's gene diversity, Shannon's information index, genetic distance D, or Nei's unbiased genetic distance, among-snail population distance of *O. h. hupensis* was greater than that between *O. h. robertsnoi* populations ($P < 0.01$). Of 18 snail populations of *O. h. hupensis,* 15 snail populations had ribbed-shells, and three snail populations had smooth shells. Nei's gene diversity and Shannon's information index among ribbed-shell populations of *O. h. hupensis* were higher than that among smooth-shelled populations of *O. h. hupensis* ($P < 0.01$), but genetic distance D and Nei's unbiased genetic distance among ribbed-shelled populations of *O. h. hupensis* were lower than that among smooth-shelled populations of *O. h. hupensis* ($P < 0.01$). If the smooth-shelled populations of *O. h. hupensis* were not included a snail population from Guangxi province, Genetic distance D and Nei's unbiased genetic distance among smooth-shelled populations of *O. h. hupensis* were not different from that among ribbed-shelled populations of *O. h. hupensis* ($P > 0.05$). Nei's gene diversity and Shannon's information index among populations of *O. h. robertsoni* were not different from that among smooth-shell populations of *O. h. hupensis* ($P > 0.05$), but genetic distance D and Nei's unbiased genetic distance among smooth-shell populations of *O. h. hupensis* were higher than

TABLE 4. Genetic variation among the populations of *Oncomelania hupensis*.

	O. h. robertsoni	*O. h. hupensis*			Total
		Ribbed	Smooth	Total	
Nei's genetic diversity	0.201 ± 0.0030	0.253 ± 0.0028	0.209 ± 0.0035	0.262 ± 0.0027	0.280 ± 0.0026
Shannon's information index	0.300 ± 0.0034	0.384 ± 0.0039	0.300 ± 0.0051	0.399 ± 0.0037	0.426 ± 0.0035
Nei's unbiased genetic distance	0.278 ± 0.0088	0.310 ± 0.0035	0.380 ± 0.0150	0.328 ± 0.0032	0.328 ± 0.0039
Nei's unbiased genetic identity	0.758 ± 0.0067	0.729 ± 0.0026	0.684 ± 0.0101	0.722 ± 0.0023	0.722 ± 0.0028
GS_{Dice}	0.797 ± 0.0055	0.765 ± 0.0022	0.722 ± 0.0064	0.759 ± 0.0020	0.761 ± 0.0024
Genetic distance D	0.203 ± 0.0055	0.235 ± 0.0022	0.278 ± 0.0064	0.241 ± 0.0020	0.239 ± 0.0024

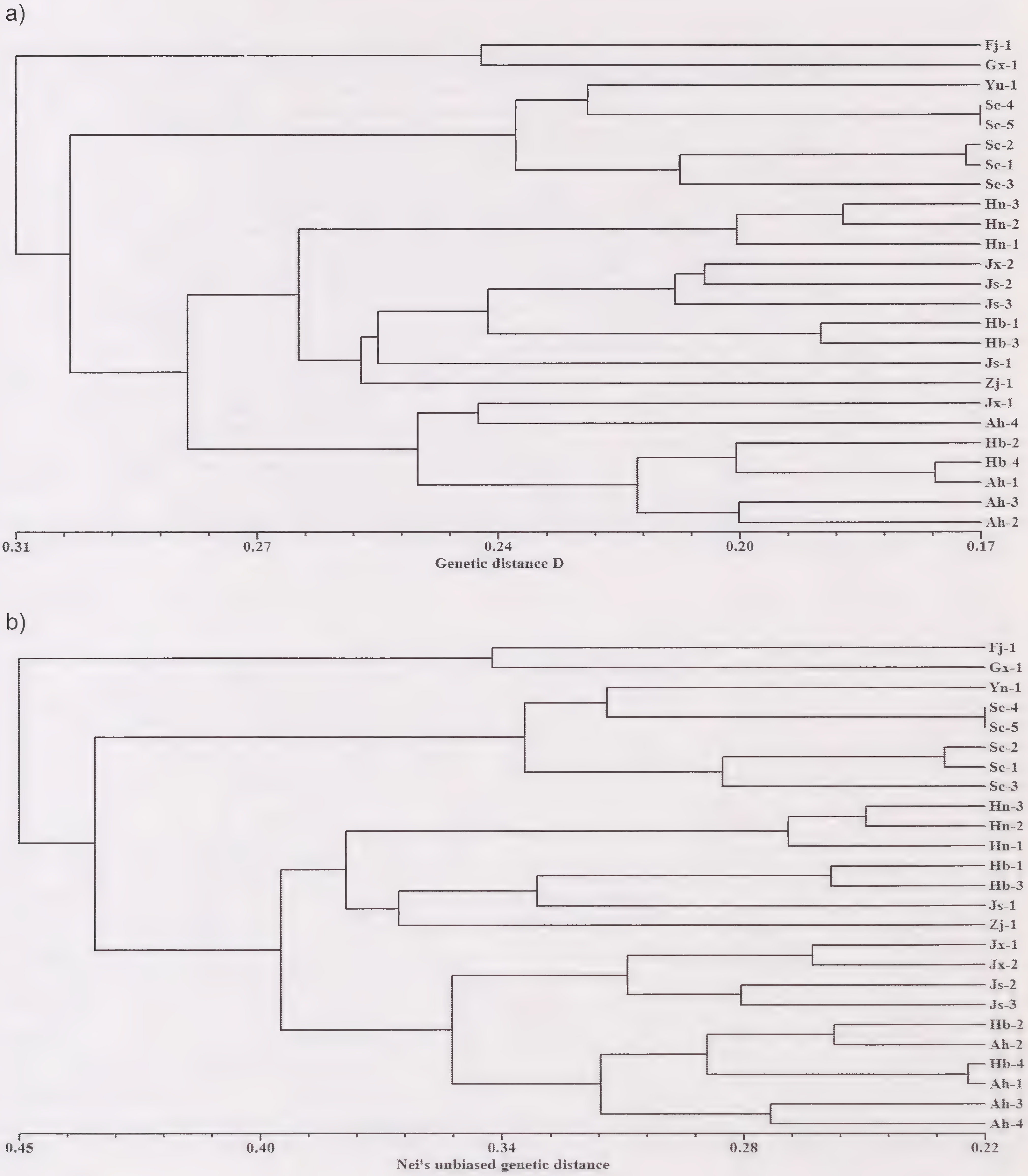


FIG. 4. Complete-link trees depicting phylogenetic relationships among twenty five snail populations of *O. hupensis* using (a) Genetic distance D matrix and (b) Nei's unbiased genetic distance matrix.

that among populations of *O. h. robertsoni* ($P < 0.01$). Whether Nei's gene diversity, Shannon's information index, Genetic distance D or Nei's unbiased genetic distance among ribbed-shell populations of *O. h. hupensis* was larger than that among snail populations of *O. h. robertsoni* ($P < 0.01$) (Table 4).

Snail AFLP Phylogeny

Analyses of Genetic distance D and Nei's unbiased genetic distance matrixes using the Complete-link method, Neighbor-joining (NJ) method, Single-link method and Unweighted pair-group method with arithmetic means

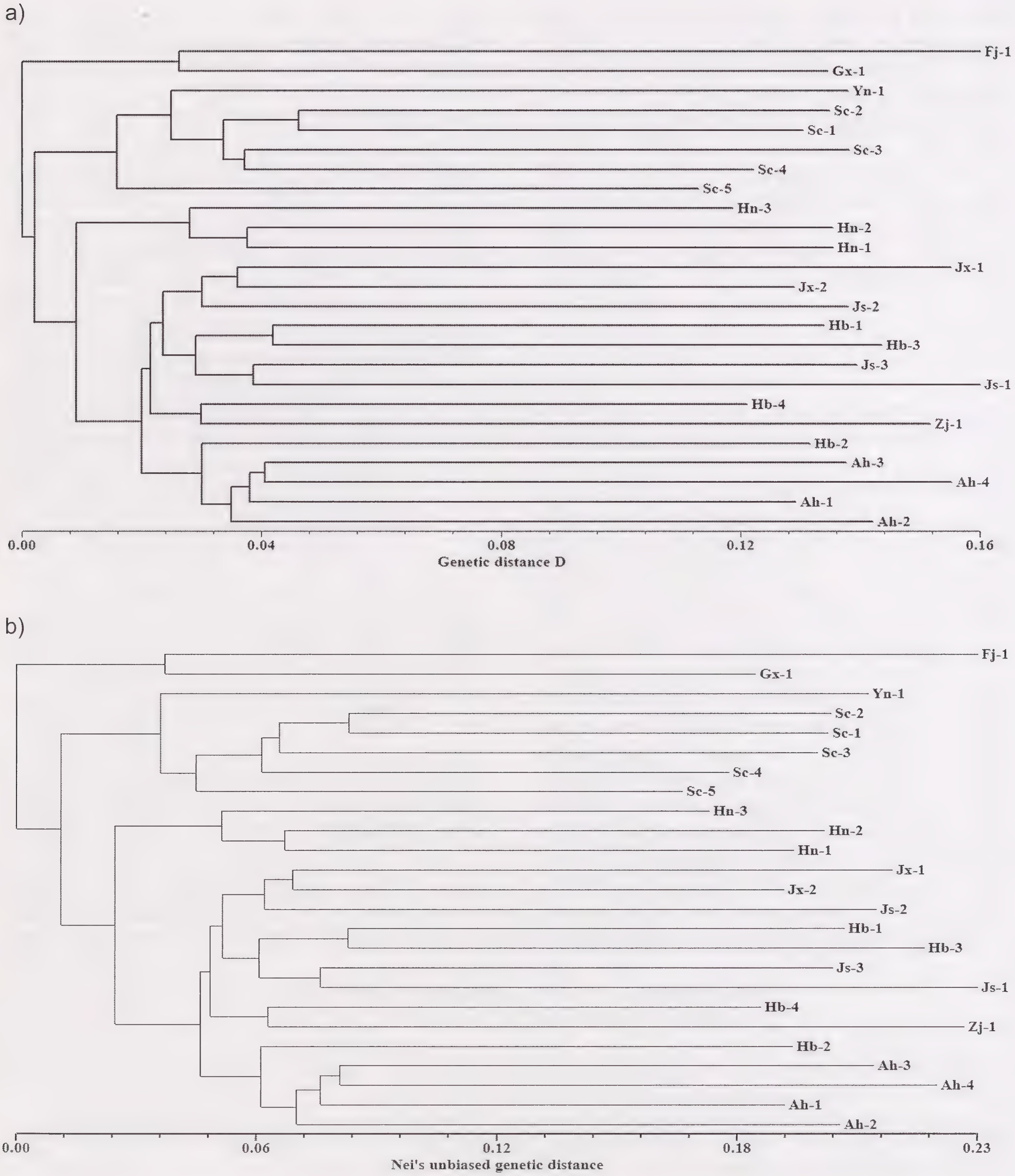


FIG. 5. Neighbor-joining trees depicting phylogenetic relationships among twenty five snail populations of *O. hupensis* using (a) Genetic distance D matrix and (b) Nei's unbiased genetic distance matrix.

(UPGMA) method respectively were shown in Figs. 4–7. For the complete-link method and NJ method, whether Genetic distance D matrix or Nei's unbiased genetic distance matrix was analyzed, the two Complete-link trees and the two NJ trees were similar to each other, and especially the NJ phylogram using Genetic distance D matrix was very similar to the NJ tree using Nei's unbiased genetic distance matrix. These four trees were all rooted with Fj-1 and Gx-1 populations and showed two big branches. One branch included six smooth-shelled populations without varix (Yn-1, Sc-1-Sc-5) from Yunnan and Sichuan Prov-

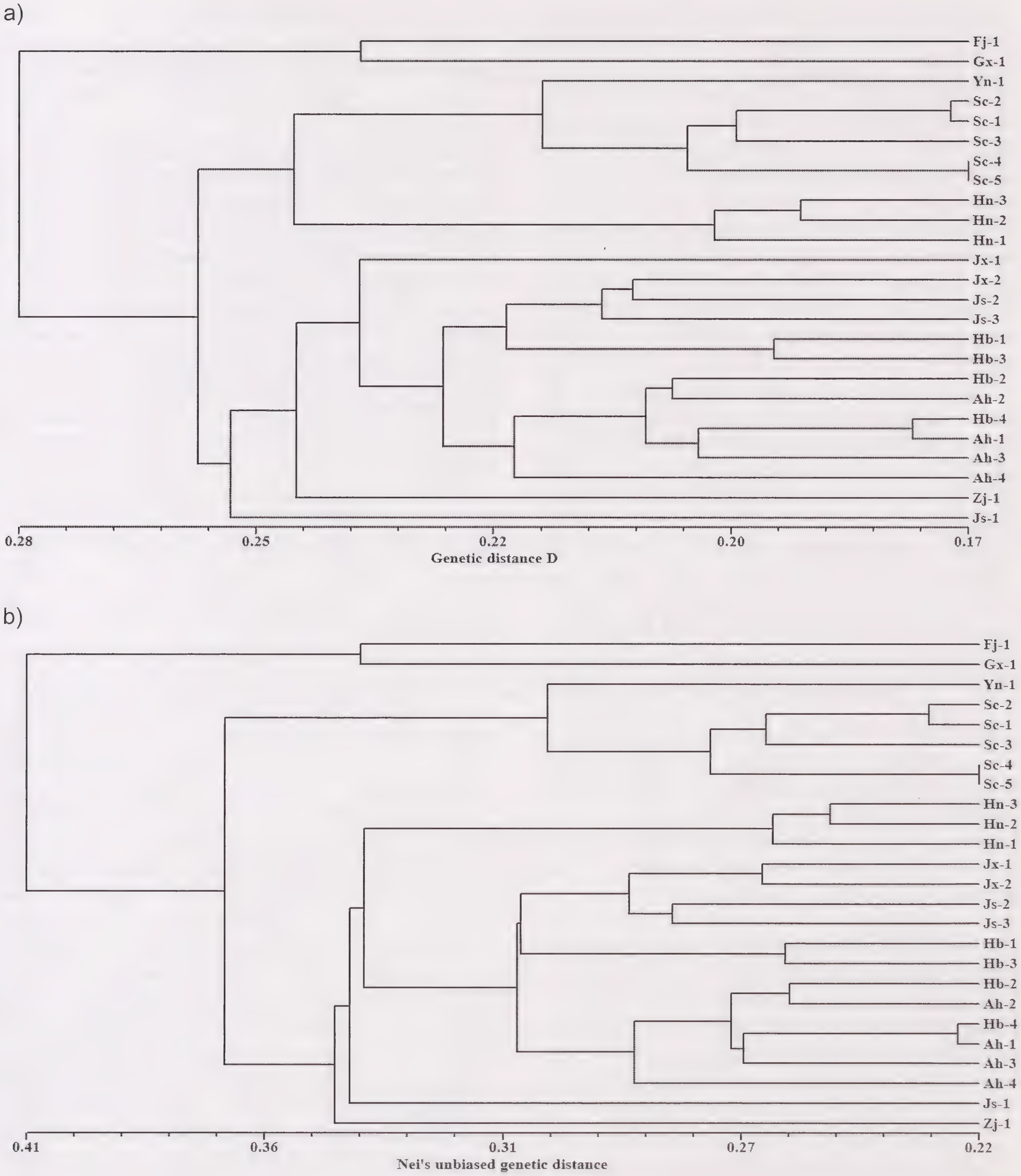
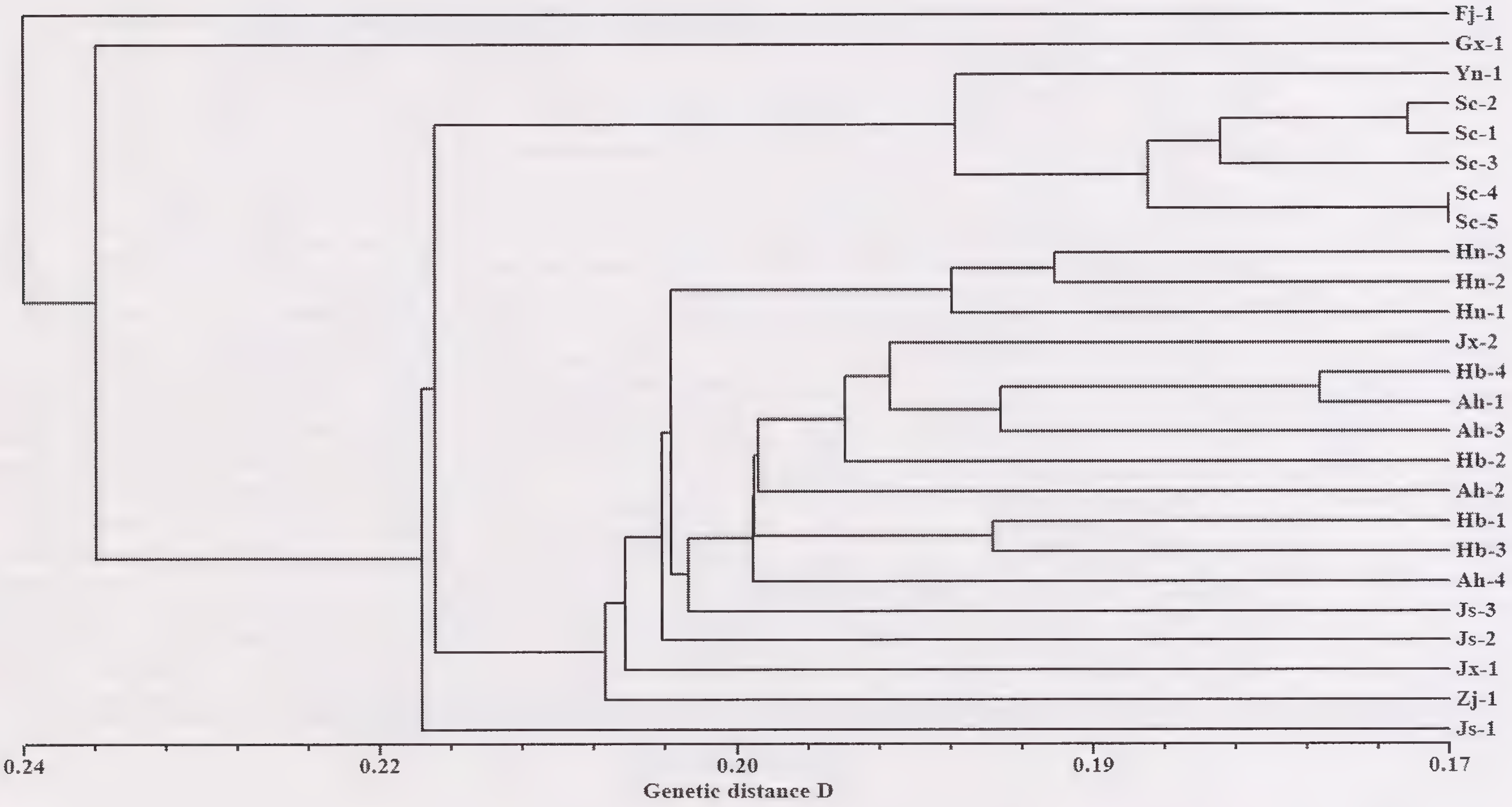


FIG. 6. UPGMA trees depicting phylogenetic relationships among twenty five snail populations of *O. hupensis* using (a) Genetic distance D matrix and (b) Nei's unbiased genetic distance matrix.

inces, the other branch consisted of other 17 snail populations from throughout the Yangtze River drainage below the Three Gorges to Jiangsu and Zhejiang provinces. The two UPGMA trees also were both similar to the four trees above, and were both rooted with Fj-1 and Gx-1 populations and showed two big branches. Unexpectedly, three ribbed-shelled populations (Hn-1, Hn-2 and Hn-3) from Hunan Province fell within the *O. hupensis robertsoni* subspecies (which is found in Yunnan and Sichuan provinces) clade in the UPGMA tree using Genetic distance D matrix, but the genetic distance D between a)



b)

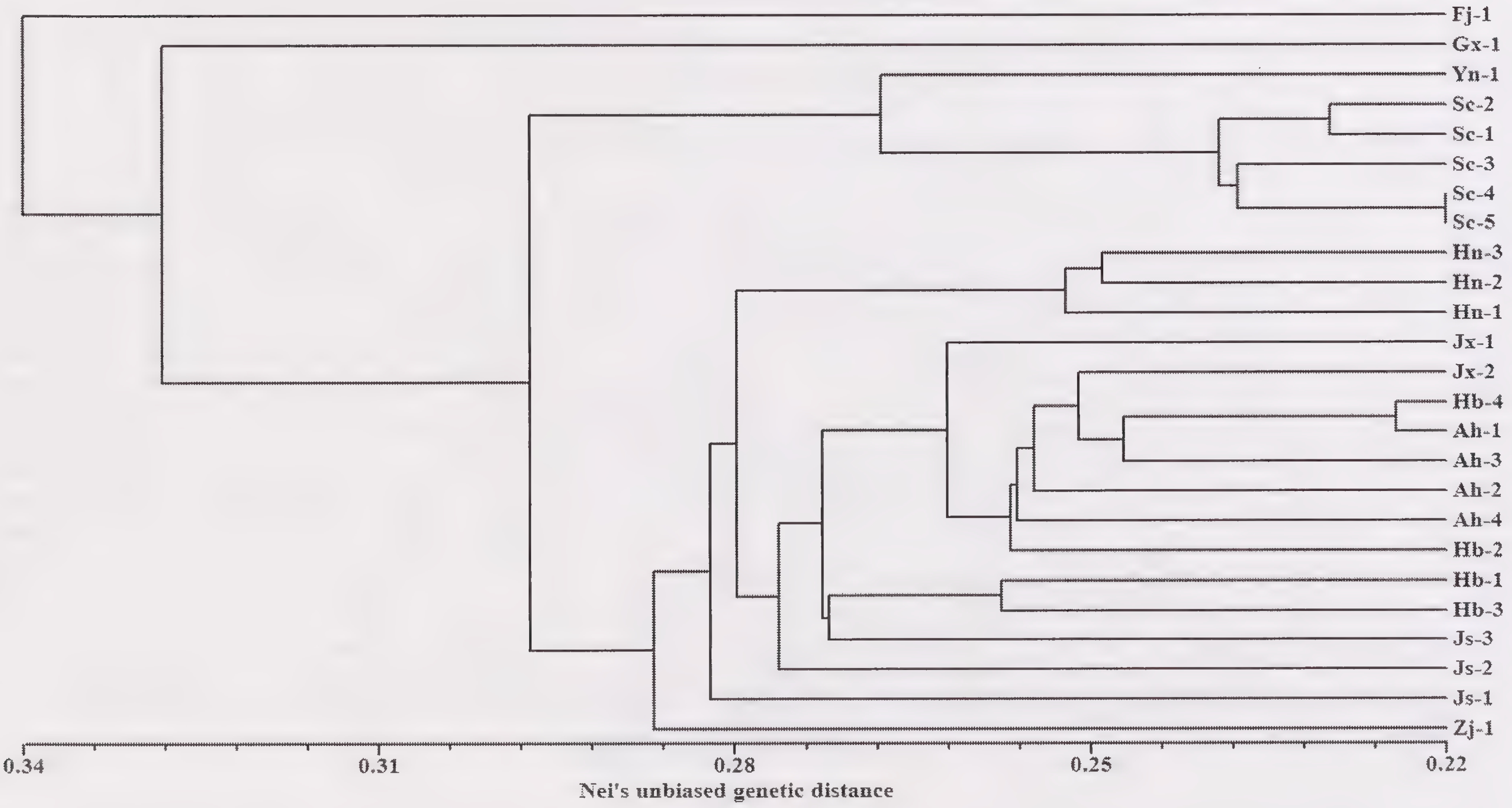


FIG. 7. Single-link trees depicting phylogenetic relationships among twenty five snail populations of *O. hupensis* using (a) Genetic distance D matrix and (b) Nei's unbiased genetic distance matrix.

the snail populations from Hunan Province and the snail populations from Yunnan and Sichuan provinces was relatively big (0.220 to 0.298). The two single-link trees were different from six other trees above, and were both rooted with only Fj-1 populations, with Gx-1 population divided into an independent branch. The other 23 snail populations also were divided similarly into two big branches except the Js-1 population in the tree using genetic distance D matrix, which was divided into an independent branch.

DISCUSSION

Appropriate Sample Size and Number of AFLP Markers

Due to intra-population genetic variation, one or several snail individuals are insufficient to analyze population variation. The snail population (Hn-2) selected to examine how much variation increased with sample size, and the number of AFLP molecular loci showed that the percentage of polymorphic loci, Nei's gene diversity, and Shannon's information index all increased gradually with increasing sample size up to a sample size of 30 or more individuals, When the sample size was less than seven individuals, the values of these indices are very unstable. Our result is consistent with the sample size calculated based on the formula of Brown & Moran (1981) in a random mating population. So we sampled randomly 30 snail individuals not infected with *S. japonicum* from a snail population to represent this population in this paper. We recognize the fact that if additional populations were added, variation would continue to increase. The practical solution, universally recognized, is to use between 15 to 25 individuals for population genetic studies.

Our AFLP analysis data still showed, on basis of 30 snails sample from Hn-2 population, that the percentage of polymorphic loci, Nei's gene diversity, and Shannon's information index change rather acutely when the number of loci is less than 128, while the values of theses indices trend to stabilization when the number of loci is more than 338. These indicate that the appropriate number of AFLP markers for studying the genetic variations of *O. h.* by AFLP method is optimally not less than 338 for each *Oncomelania* population, whereas the result is very unreliable when the number of AFLP markers is less than 128. In our study, the total eight primer pairs were used to analyze genetic diversity of *O. hupensis*, and the number of AFLP markers amplified was in range of 403–467 bands for the 13 *Oncomelania* populations. So it was enough and reasonable to use only eight primer pairs for studying and comparing the genetic variation of difference *Oncomelania* populations.

Intra-Population Genetic Diversity

In a previous allozyme study of *O. hupensis*, Davis et al. (1995) found that 14 snail populations from Mainland China had few alleles per locus (1.0 to 1.6), a low mean heterozygosity (0.008 to 0.036), and low percentage polymorphic loci (4 to 28). In another previous COI gene sequencing study of *O. hupensis*, Wilke et al. (2000) also found relatively little genetic variability within the in-group individuals, and the nucleotide diversity ranged from 0 to 0.0196. Compared with these relatively conservative allozyme and COI marker, our AFLP data showed that there was higher intra-population genetic variation in mainland China – the percentage of polymorphic loci ranged from 55.80% to 93.22%, the Nei's gene diversity from 0.191 to 0.345, and the Shannon's information index from 0.287 to 0.510 – furthermore, these intra-population genetic variations from different areas differed significantly. These substantial differences were present among not only the three subspecies, but also different populations of the same subspecies, especially *O. h. hupensis.* The results found in our study were similar to those by Wilke et al. (2006), and they found each snail individual had a unique AFLP fingerprint in the preliminary AFLP analysis of 24 specimens. So our results might indicate that AFLP approach can examine more genetic diversity of *O. hupensis* than allozyme and COI gene sequence.

Oncomelania hupensis tangi has been eradicated except for two known populations, and the parasite presumably is extinct. Our AFLP data showed interestingly that the genetic variation within Fj-1 from *O. h. tangi* as well as Gx-1 population was very low. *Schistosomiasis japonicum* in Guangxi Province has been exterminated, except for intermediate snail hosts restricted in narrow areas. This might be mostly due to the fact that these snail populations are localized relatively narrow areas, and the number of individuals in these snail populations is very limited, so the genetic drift can easily happen in these snail populations, and the results of the genetic drift will lead to farther decrease of their genetic diversity.

Inter-Population Genetic Diversity and Phylogeny

Our AFLP analysis data from 25 snail populations show that considerable genetic differentiation has been occurred throughout China. Genetic distance D was from 0.169 to 0.306, and Nei's unbiased genetic distance from 0.225 to 0.452. These two indices of genetic distance with the four clustering methods were used to reconstruct the phylograms of *O. hupensis* from mainland China, and most results of the eight phylogenetic trees were similar, and patterns of genetic differentiation were in general correlated with geographic distribution. These results were similar to the results of allozymes and COI gene sequences (Davis et al., 1995, 1999a, b; Wilke et al., 2000; Shi et al., 2002) excluding a snail population from Guangxi Province (Gx-1). These patterns thus suggested that there were three distinct clades of *O. hupensis* on mainland China.

The six snail populations from Yunnan and Sichuan provinces, namely *O. h. robertsoni*, were less, and they always fell within a clade in eight phylograms. This supported that snails from Yunnan and Sichuan provinces belong to the same subspecies, *O. hupensis robertsoni*.

Excluding in two single-link trees, Fj-1 and Gx-1, populations always fell within a clade in other six trees. This was dissimilar to the result of allozyme studies (Davis et al., 1995), namely our AFLP analysis data did not support that the Gx-1 population belongs to *O. hupensis hupensis*. In fact, the Gx-1 population is far from the other populations of *O. h. hupensis*, and no snail populations have also been found in the areas between them. The genetic distance D between Gx-1 and Fj-1 snail populations was 0.240, and Nei's unbiased genetic distance was 0.342. There are some morphological similarities between Fj-1 and Gx-1 populations: the shells of these snails are smooth with a varix, and proportionally wider for their length. But the geographical distance between the two snail populations is far, and the shells of Gx-1 snails are thin with weak varix compared with that of Fj-1 population. The genetic distance between Fj-1 and Gx-1 populations is an average level of that among 25 populations. So, should the snails from Guangxi Province be an independent subspecies (i.e., *O. hupensis guangxiensis*) according to the classification of Liu et al. (1981), or be incorporated with snails from Fujian Province into a subspecies? This need further study.

Among the other 17 snail populations, the Js-1 population was an independent clade in the single-link tree using genetic distance D matrix, and the three ribbed populations (Hn-1, Hn-2 and Hn-3) fell within the *O. hupensis robertsoni* subspecies clade in the UPGMA tree using genetic distance D. matrix. However, these snail populations always fell within a clade in the other six trees. These results were similar to the results of allozyme and COI gene sequence, and showed the 17 populations are a subspecies, *O. hupensis hupensis*.

Our results still show the genetic variation between snail populations of *O. h. hupensis* is greater than that between populations of *O. h. robertsoni*. This might indicate there is bigger divergence among *O. h. hupensis*, compared with *O. h. robertsoni*. However, Wilke et al. (2006) found that in their study of 66 individuals from 13 populations of *O. h. robertsoni*, that genetic divergence, using combined COI/LSU rRNA, was an order of magnitude greater than among populations of *O. h. hupensis* (0.042 and 0.085 for different lineages of *O. h. robertsoni;* 0.0097 for other *O. h. subspecies.* But when AFLP was used on 24 individuals, each had a unique fingerprint.

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SEASONAL CHANGES IN COLD HARDINESS OF THE INVASIVE FRESHWATER APPLE SNAIL, *POMACEA CANALICULATA* (LAMARCK) (GASTROPODA: AMPULLARIIDAE)

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ABSTRACT

We investigated the cold hardiness of a South American freshwater apple snail, *Pomacea canaliculata*, which began to invade Japanese paddy fields in the early 1980s. *Pomacea canaliculata* exhibited apparent seasonal fluctuation in its cold hardiness. Snails collected from submerged paddy fields in summer were less tolerant to cold, and none survived exposure to 0°C for five days. With decreasing temperature, together with drainage of its habitat in autumn, *P. canaliculata* developed cold hardiness, attaining almost 100% survivorship after exposure to 0°C for five days in December. The snails resting in drained fields were approximately nine times more cold tolerant than those collected from submerged fields, based on the time to 50% mortality at 0°C. Snails overwintering in aquatic conditions also acquired cold hardiness.

Key words: apple snail, cold hardiness, invasion, overwintering, rice field, *Pomacea canaliculata*.

INTRODUCTION

The apple snail *Pomacea canaliculata* (Lamarck, 1819), often referred to as the golden apple snail in Southeast Asia, was introduced into Japan and many other countries in Southeast and East Asia in the early 1980s (Mochida, 1991; Litsinger & Estano, 1993). This freshwater snail, originating from South America, was initially introduced as a source of human food. However, commercial markets failed, and discarded or escaped snails invaded rice ecosystems, becoming a serious pest of rice in many countries (Naylor, 1996; Wada, 2004). *Pomacea canaliculata* is naturally distributed in a wide area from the Pantanal, Brazil, to 38°S in Argentina, mostly inhabiting shallow lakes, ponds, swamps and the catchment areas of rivers (Halwart, 1984; Martin et al., 2001). The origins of Asian *P. canaliculata* are unknown, but may include many sources.

In Japan, *P. canaliculata* mostly inhabits paddy fields and irrigation canals. In paddy fields, *P. canaliculata* shows explosive population growth every year. In Kumamoto, southern Japan, normal season rice is planted in June. Before rice planting, drained paddy fields are irrigated for harrowing a few days before the seedlings are planted. This irrigation breaks the dormancy of snails that have overwintered there. The overwintered population of the snails comprises a majority of juveniles of intermediate size (shell height approximately 7–18 mm) (Watanabe et al., 2000; Wada et al., 1999) that were born in the previous crop season, and very small number of adult snails, most of which seem to have survived through the two previous crop seasons. The total snail densities are usually not high, for example 2.8 ± 1.1 m^2 (mean ± S.E.) in paddy fields of northern Kyushu in 2000 (Wada et al., 2004). The adult snails soon begin sexual activity, and females lay egg masses in the fields. The adults grow very slowly, probably because of heavy investment in reproductive activity. On the other hand, the juveniles grow very rapidly, becoming adults in a month or so after paddy irrigation. Thus, the two types of snails present at irrigation overlap in size, and cannot be separated on that basis in July. Almost simultaneously, juveniles of the new gen-

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eration appear in paddy fields. Thus, again, two types of snails in terms of size constitute the snail population in summer (Syobu, 1996). Snails tend to stop growing after August, due to a paucity of food (Tanaka et al., 1999). However, population densities gradually increase due to continuous appearance of the new generation during the irrigation period. By early autumn, the population densities often reach a few hundred/m^2 (the majority being juveniles smaller than 10 mm), which is sometimes more than 100-fold the initial overwintered population (Wada et al., 2004). This population explosion is attributed to the lack of natural enemies in paddy fields (Yusa et al., 2006). Most juveniles of the new generation do not reach adulthood until drainage of the paddy fields in late September before the rice harvest. When farmers stop irrigation, many or most of the snails bury themselves in the soil and enter dormancy prior to overwintering. However, many snails fail to bury themselves, and instead overwinter under straw scattered on the fields at harvest time in mid October. Mortality of adult snails in winter is higher than that of juveniles of intermediate size, because intermediate-sized snails are more tolerant to the cold (Syobu et al., 2001; Oya et al., 1987), and also the tillage sometimes carried out by farmers during the fallow season kills large snails (Takahashi et al., 2002). Therefore, it seems difficult for *P. canaliculata* individuals to survive more than three crop seasons in paddy fields. In comparison with paddy snails, the ecology of *P. canaliculata* inhabiting irrigation canals has been scarcely studied, probably because of the difficulty in tracing populations. Very large snails are often found in canals, and therefore some snails in this environment appear to have a much longer life span than paddy snails.

Winter mortality of this invasive snail apparently constrains its distribution and reproduction in Japan. The northernmost known population of the snail in Ibaraki, central Japan, cannot overwinter in paddy fields, which are recolonized every year by snails that have overwintered in a limited area in canals (Ito, 2002). Even in Kyushu, southern Japan, the snail is distributed mainly in lowland areas and cannot invade paddy fields in mountain areas, where the temperature decreases considerably in winter. Winter mortality of the hibernating snails in paddy fields reportedly varies. In Kyushu, estimated mortality has been reported to be 65–95% in Chikugo (Oya et al., 1987) and 85% in Kumamoto (Watanabe et al., 2000), and 18–67% mortality has also been reported for snails hibernating under rice straw in Saga (Syobu et al., 2001). In Shizuoka, which has a generally warmer winter, mortality in winter has been estimated to be less than 40% (Ozawa & Makino, 1988). However, these figures probably underestimate mortality, because small snails, which usually comprise the majority of the snails in autumn paddy fields and are less tolerant of cold weather (Syobu et al., 2001; Oya et al., 1987), were often not included in the data. In fact, Wada et al. (2004), who carefully investigated population densities including very small snails, reported that the difference in densities between in early summer and autumn fields was 60- to 940-fold. Thus, the populations of *P. canaliculata* in Japan collapse during winter, and this is a key constraint on population growth of the invasive snail in its new habitat in temperate Japan.

A few studies have experimentally examined the cold hardiness of *P. canaliculata* in Japan, and generally these have demonstrated that the snail is not tolerant of cold temperatures. For example, laboratory-reared progeny of snails collected in four locations in Japan and the Philippines mostly died within three days at 0°C, irrespective of their origin (Sugiura & Wada, 1999). Ohgami (1986) also reported that most snails died after several days at 2°C. However, according to Oya et al. (1987), snails collected in winter survived more than ten days at 0°C. Thus, it seems that there is some degree of discord among previous studies with respect to the cold hardiness of *P. canaliculata*.

In this study, we attempted to clarify the reason for this discord by sampling the snails periodically from fields. There is a possibility that *P. canaliculata* has evolved to enhance cold hardiness in Japanese paddy fields, because it has been selected by cold Japanese winters for more than 25 years. In addition, it is important for us to know winter mortality of the snails and its mechanism so as to develop a new management suppressing the population of pest snails during winter.

MATERIALS AND METHODS

Snails Used

The snails used were collected from paddy fields in Kikuchi, Kumamoto Prefecture (32°57'N, 130°45'E), except as indicated below. We collected active snails from submerged paddy fields during summer and snails resting under straw from drained fields in autumn and winter. When we collected snails from submerged fields, we were able to discriminate live snails from dead ones by the presence of an operculum. Under aquatic conditions, the operculum of a dead snail soon disappears from the shell. However, under dry conditions, since a dead snail retains its operculum for many days, we were unable to discriminate live snails when collecting in drained paddy fields. Therefore, we had to examine snail mortality using the method described below, while simultaneously testing for cold hardiness. When the initial mortality was not zero, we applied Abbott's correction (Abbott, 1925) to the results of the cold hardiness tests.

Differences in Cold Hardiness According to Size

Since few reports (Syobu et al., 2001; Oya et al., 1987; Watanabe et al., 2000) have documented differences in cold hardiness of *P. canaliculata* according to size, we first evaluated such differences in detail. We collected snails on September 15, 2005, from a few submerged paddy fields. The snails were washed with tap water and then left at room temperature until dry. Then, the snails were divided into five classes according to size and sex: very small juveniles with shell heights of 5–7.5 mm, small juveniles of 7.6–12.5 mm, juveniles of 12.6–20.0 mm, adult males (shell heights exceeding 23.0 mm) with peculiarly shaped opercula and apertures (Cazzaniga, 1990), and adult females with shell heights larger than approximately 25.0 mm, since females mature at this size (Tanaka et al., 1999). Thirty snails of each size class (25 snails for adults) wrapped with wet paper were confined in a plastic container (11 cm x 9 cm, 4 cm in depth for juveniles, and 19 cm x 12 cm, 6 cm in depth for adults) and kept in an incubator for two days at temperatures of 3°C, 0°C, -3°C and -5°C (± 1.0°C). Two replicates were carried out. After incubation, we discriminated live snails from dead ones as described below.

Seasonal Variation

To examine seasonal fluctuation in the cold hardiness of *P. canaliculata*, we periodically collected juveniles from a paddy field from June 2005 to March 2006. The shell heights of the snails were fixed from 7.5 to 17.5 mm (adult snails were collected only on December 7). On some occasions, we randomly selected 50 to 100 snails from the collected samples and measured their shell heights with calipers to monitor snail size. The first sampling occasion was on June 21, about a week after initial irrigation of the field for harrowing. Thus, the samples were overwintered snails that had broken their dormancy. From the second sampling occasion onwards, the snails sampled were the progeny (first generation) of the overwintered snails (see Introduction). Snails collected when the paddy field was drained (samples of October and thereafter) were checked for mortality, while cold treatment tests were simultaneously conducted. For cold treatment, 25 to 60 snails were wrapped with wet paper and kept in a plastic container (11 cm x 9 cm, 4 cm). They were then incubated for two days at 3°C, 0°C, -3°C, and -5°C, and for five days at 0°C (two replicates). In January 2006, survival of field snails declined considerably, and therefore it became difficult to carry out the cold treatment tests thereafter.

On March 8 and 15, we sampled the paddy soil containing hibernating snails to estimate roughly the mortalities and densities of the snails that had successfully buried themselves. First, we sampled a 0.25 m^2 area of soil (0.5 m x 0.5 m, 0.1 m depth) at 12 locations selected approximately randomly in the field after removing straw and snails on the soil surface. We brought the soil to the laboratory and carefully removed the snails from the soil by washing the soil away through a fine-mesh metal bowl. We then measured the size of the snails and checked whether they were alive. Similarly, we collected all snails resting under straw from a 0.25 m^2 quadrate at 16 locations in the field and estimated the mortality and density of the snails. We conducted further sampling of snails under the straw in additional areas of the field to increase the sample size. The March 8 and 15 data were combined, because the area of soil sampled on each occasion was too small.

Temperatures under the rice straw and on the soil surface without straw were monitored with data loggers every hour from December 8 to March 14. We also used data measured by the Japanese Meteorological Agency at the AMeDAS (Automated Meteorological Data Acquisition System) point at Kikuchi, 3 km from the survey field, as a substitute for air temperatures at 1.2 m above the soil surface.

Survivorship at 0°C

We compared survivorship at 0°C of snails collected in summer and those collected in winter. Hibernating snails were collected from a drained paddy field on January 8 and 18, 2005. Summer snails were collected from submerged paddy fields on July 21 and August 8, 2006. The shell heights of the snails collected ranged from 7.5 to 12.5 mm. Thirtyfour to 60 snails in each treatment period, wrapped with wet paper were kept in a container (11 cm x 9 cm, 4 cm), and incubated at 0°C for various periods (no replicate). After incubation they were subjected to the mortality test.

Cold Hardiness of Overwintering Snails under Aquatic Conditions

We collected overwintering snails from a concrete water tank placed outdoors in Kyushu National Agricultural Research Center on February 18, 2005, to examine the cold hardiness of overwintering snails under aquatic conditions. The snails were a stock culture that had originated from paddy fields in Kikuchi. The shell heights of snails collected ranged from 7.5 to 12.5 mm. Forty snails in each treatment were wrapped with wet paper and kept in a container (11 cm x 9 cm, 4 cm) (two replicates). They were then incubated at 0°C for five days and ten days and subjected to the mortality test.

Mortality Test

For the snails collected from drained fields and the snails that had been subject to cold tolerance treatments, we recorded the proportions of individuals that survived (mortality test). The snails were immersed in tap water in a glass container and left at room temperature (20°C to 28°C) for approximately one day. If the snails were alive, they opened their opercula and extruded their soft parts. Therefore, we considered snails that extruded the foot and/or tentacles from the shell within a day as alive. Some snails half-opened their opercula and responded by closing them when probed with a forceps. We regarded such snails as dead because most exhibiting this type of response finally died.

Statistical Analysis

In this study, the experiments were conducted basically in a two-replicate design. The proportions of snails that survived after cold treatment attained generally similar values between replicates, except for the treatments at -3°C. Therefore, we combined the data from the two replicates, calculating the net proportions of survivorships for each treatment. Then, analysis of multiple comparisons of proportions was carried out according to the method of Zar (1996).

To obtain the LT_{50} (time until 50% of the snails died) at 0°C, we used the regression of the proportions of surviving snails against treatment time (days). The percentage data were transformed into prohibits before regression calculation.

RESULTS

Differences in Cold Hardiness According to Size

Juveniles of intermediate size were more tolerant of cold temperature than “very small juveniles” and adults (Fig. 1). This was apparent when snails were kept at 0°C for two days. More than half of the “small juveniles” and “juveniles” survived, while the survivorships were about 10% for “very small juveniles” and adults, the differences being significant. At -3°C and lower temperatures, no snails except for a juvenile survived for two days. No difference in cold hardiness was found between the sexes. However, when considering survivorships at 3°C within each sex, bigger males were more cold tolerant than smaller ones, that is, the average shell height of viable males (mean ± S.D. 30.5 ± 3.3 mm) was significant larger (*t*-test, $P < 0.001$) than that of dead snails (25.9 ± 1.8 mm). This was not the case for females (alive, 31.7 ± 2.5 mm; dead, 31.7 ± 3.2 mm, *t*-test; $P > 0.9$).

Survivorship of Field-Collected Snails and Changes in Cold Hardiness

A majority of the juveniles collected up to December 2005 under straw in the field were alive (Table 1), but by January 2006, only 27% of juveniles under the straw were alive. By mid March, only 1.4% were alive. In mid March, we sampled snails hibernating in the soil as well as those under straw. In the soil, 7.8% of the juveniles (7.5–17.5 mm) were alive, which was significantly higher than for juveniles under straw (Fisher’s exact probability test, $0.001 < p < 0.01$). Juveniles smaller than 7.5 mm were all dead, both in the soil and under straw. The roughly estimated densities of live and dead very small juveniles (< 7.5) and juveniles

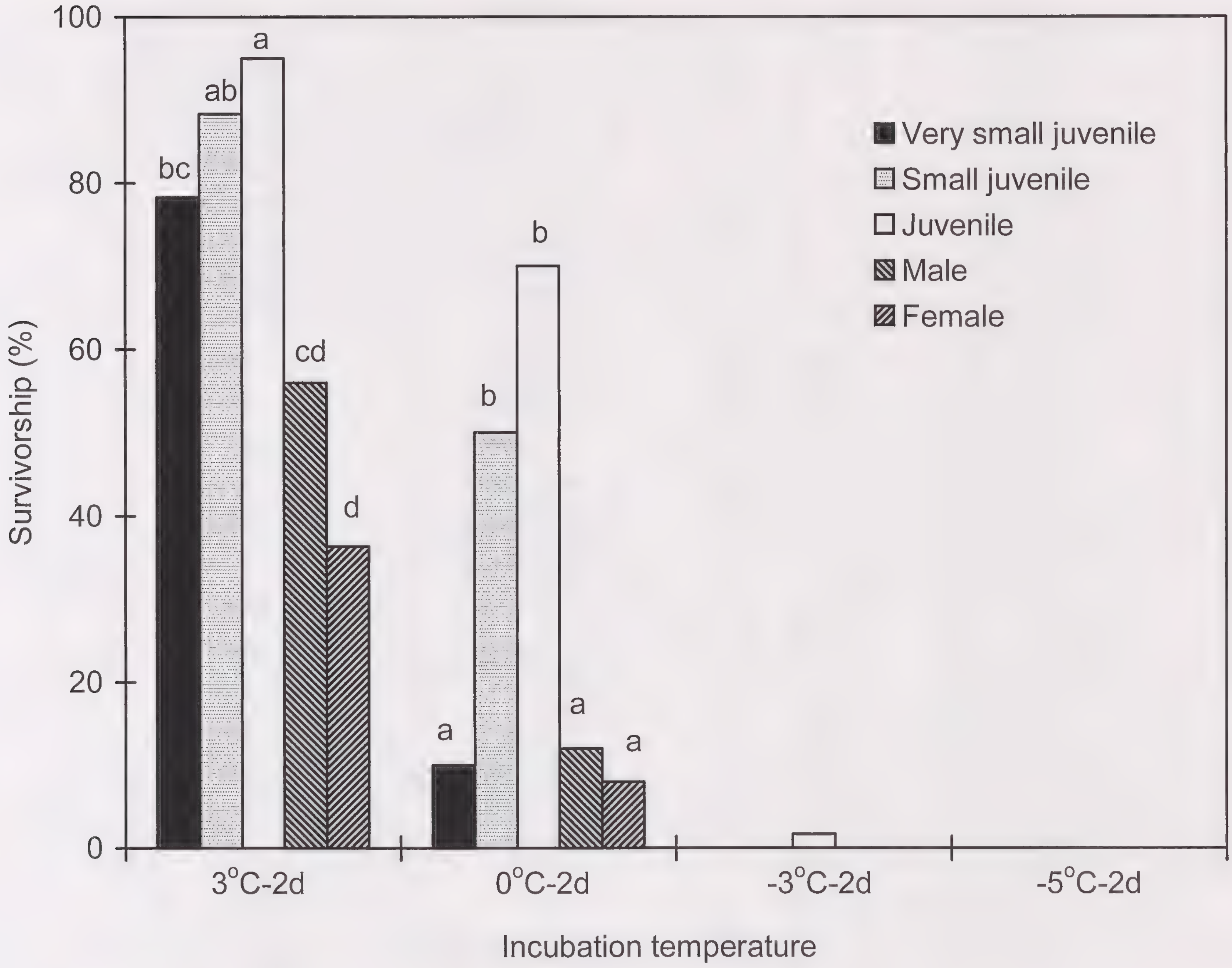

FIG. 1. Survivorships of *Pomacea canaliculata* juveniles of different sizes exposed for two days at various low temperatures. Very small juveniles, 5.0–7.5 mm; Small juveniles, 7.6–12.5 mm; Juveniles, 12.6–20.0 mm in shell height. Male: male adult, Female: female adult.

(7.5–17.5 mm) in the soil were 26.0/m^2 and 34.5/m^2, respectively, about twice those of snails under straw (11.0 and 17.8/m^2, respectively), although the densities of very small snails might have been underestimated because of the difficulty in finding them. Very few snails larger than 17.5 mm were found in either the soil or under straw.

In the cold treatments, most juveniles survived two days at 3°C, irrespective of sampling season (Table 1). At 0°C for two days, the proportions of surviving juveniles varied. The lowest survival rate (20%) was recorded for progeny of overwintered snails collected on August 6, during the hottest season of the year. Overwintered snails collected in June and their progeny collected in September, both from submerged paddy fields, showed comparatively low survival rates. On the other hand, almost all snails collected in autumn and winter from the drained field survived this cold temperature treatment, implying a seasonal fluctuation in the cold hardiness of *P. canaliculata*. At -3°C for two days, considerable numbers of juveniles collected on October 13 and December 7 survived. However, these data varied widely among replicates (containers), despite the identical incubation conditions. For example, the survivorships of two replicates were 4% and 76% (average 40%) in the samples taken on October 13, and 2.5% and 100% (average before Abbott's correction 51%) in the samples taken on December 7. Because -3°C is perhaps close to the supercooling point of the snail, the great variation among the replicates may have been because of accidental inoculative freezing.

Seasonal fluctuation in cold hardiness was conspicuous when snails were kept at 0°C for five days. No juveniles collected in summer survived, whereas the survivorships increased from October and went up to almost 100% in December. The adult snails collected on December 7 also showed a high survival of 80% (24 out of 30 sampled) when exposed to 0°C for five days. Thus, seasonal fluctuation in cold hardiness was apparent in *P. canaliculata* inhabiting paddy fields in Kumamoto. In January, a corrected value of 37% survivorship after exposure to 0°C for five days was recorded. However, this value was unreliable because the initial survivorship (27%) of the samples

TABLE 1. Survivorship of juvenile *Pomacea canaliculata* collected in fields after experimental exposure to low temperature. *Within a column, percentages followed by the same letter are not significantly different at the 5% probability level, according to analysis of multiple comparisons of proportions (Zar, 1996). **The snails collected from submerged fields were apparently alive, and thus no mortality test was examined. When the survivorship of field-collected snails was not 100%, the data were corrected by the method of Abott (1925). ***Numbers of snails tested in each cold treatment. ****On this occasion juveniles were collected in the soil of the drained paddy fields; all other snails were collected under rice straw.

		Size of snails	Survivorship of field snails		Survivorship (%)* after incubation at					
Collection date	Field condition	Mean ± S.E. (Range)	No. tested	Survivorship (%)**	No. tested***	3°C-2d	0°C-2d	-3°C-2d	-5°C-2d	0°C-5d
2005										
June 21	submerged	(7.5–17.5 mm)		(100)	60	96.7 ab	48.3 b	5.0	0	0 a
Aug. 6	submerged	11.8 ± 0.2		(100)	60	93.3 ab	20.0 a	0	0	0 a
Sep. 15	submerged	12.1 ± 0.4		(100)	120	91.7 a	60.0 b	0.8	no data	0 a
Oct. 13	drained	11.0 ± 0.3		no data	50	98.0 ab	98.0 c	40.0	0	44.0 b
Nov. 8	drained	10.6 ± 0.2	50	98.0	80	100 b	98.8 c	0	0	50.0 b
Dec. 7	drained	11.0 ± 0.2	104	96.2	80	100 b	100 c	51.3	0	97.5 c
Dec. 20	drained	(7.5–17.5 mm)	100	82.0						
2006										
Jan. 11	drained	(7.5–17.5 mm)	118	27.2	80					36.7
March 8 and 15	drained	(7.5–17.5 mm)	422	1.4						
March 8 and 15	drained	(7.5–17.5 mm)	115****	7.8						

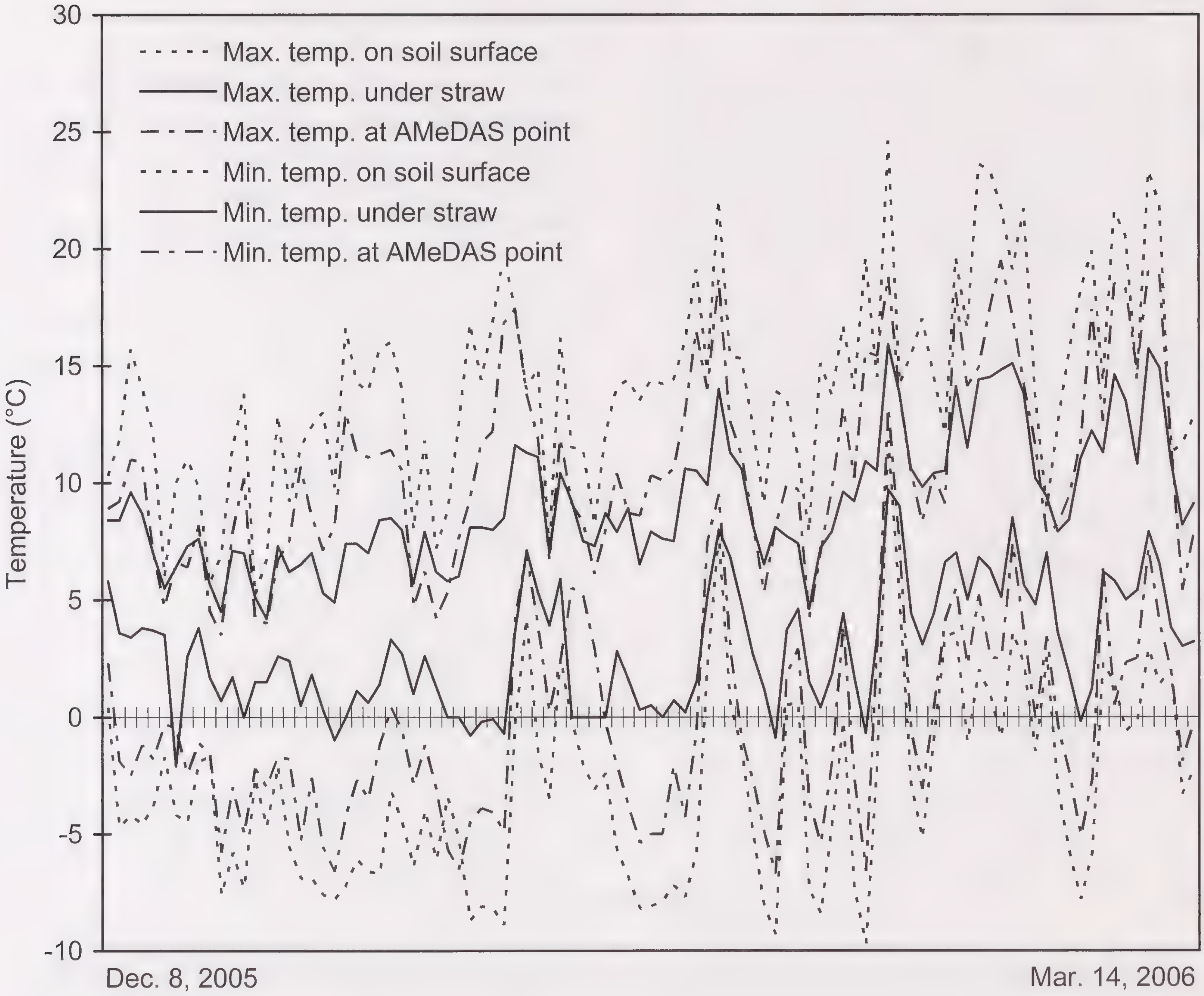

FIG. 2. Daily maximum and minimum temperatures measured on the soil surface, under rice straw, and at the nearest AMeDAS (Kikuchi) point from the survey field.

was too low for Abbott's correction. After this, we discontinued the cold treatment test, because the mortality of field snails was too high.

Cold weather was prevalent in Kyushu in the winter of 2005–2006. Nevertheless, the environment under the rice straw was well buffered thermally, and the temperature scarcely fell below zero (Fig. 2). Daily minimum temperatures under the straw were, on average, 5.0°C and 1.6°C higher than those on the soil surface and at the AMeDAS point, respectively. Similarly, daily maximum temperatures under the straw were 6.0°C and 3.3°C lower than those at the other two points, respectively.

Survivorship at 0°C

Many snails collected in January from the drained field survived more than 10 days with exposure to 0°C (Fig. 3). The LT_{50} value calculated from the regression ($Y = -0.21X + 2.46$, $r^2 = 0.89$, Y: probit of percentage mortality, X: days after 0°C incubation) was 11.9 days. On the other hand, all snails collected in July and August from the submerged fields died within three days at 0°C, having a much shorter LT_{50} of 1.3 days ($Y = -2.07X + 2.69$, $r^2 = 0.89$). Based on the LT_{50} values, winter snails were about 9.2 times more tolerant to the cold temperature than summer snails.

Cold Hardiness of Overwintering Snails under Aquatic Conditions

The snails overwintering outdoors under aquatic conditions showed survivorships at 0°C of 67.5% (± 7.5; S.E.) and 22.5% (± 7.5) for five and 10 days, respectively. The values for five days representing the extent of cold hardiness were almost comparable to those for the snails collected in winter from the drained paddy field, and were higher than those for the snails collected in summer from the submerged fields. Thus, the snails overwintering under aquatic conditions also developed cold hardiness.

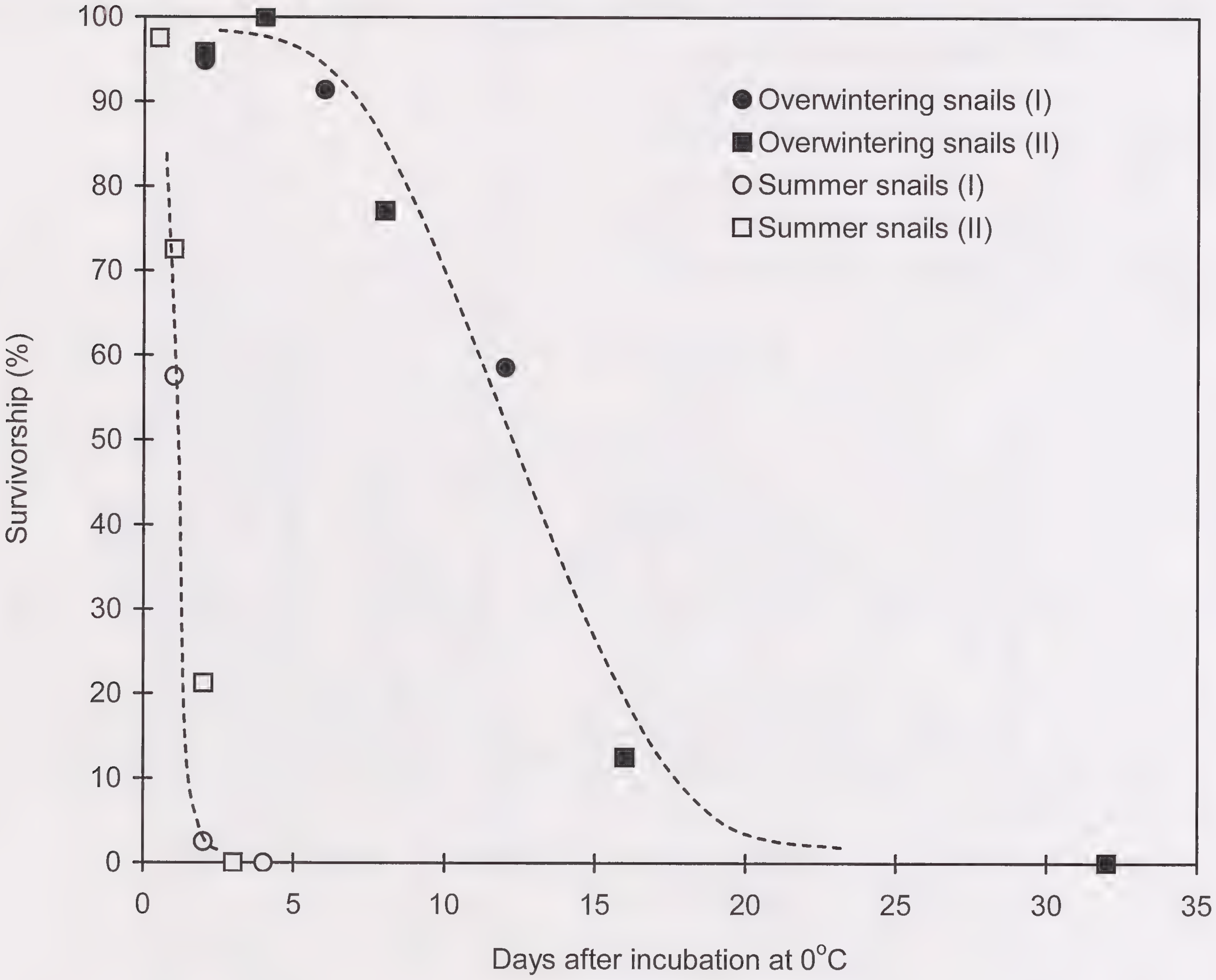

FIG. 3. Differences in survivorships after exposure to 0°C between juveniles of *P. canaliculata* collected in winter and in summer. The overwintering snails were collected from a drained paddy field on January 8 (I) and on January 18, 2005 (II). The summer snails were collected from submerged paddy fields on July 21 (I) and on August 8, 2006 (II).

DISCUSSION

We observed apparent seasonal fluctuation in the cold hardiness of *P. canaliculata*. Summer snails were less tolerant to cold even at temperatures above zero. With decreasing temperature, together with drainage of its habitat, *P. canaliculata* developed cold hardiness. Such environmental conditions enhanced the degree of cold hardiness (Matsukura & Wada, submitted). Snails overwintering under aquatic conditions also acquired cold hardiness in winter.

The strategies adopted by ectotherms for overcoming winter cold have been frequently reviewed (for example, Storey & Storey, 1997; Ansart & Vernon, 2003). The three main strategies are: moving to a favorable habitat (behavioral freezing avoidance), avoidance of freezing by extensive supercooling (physiological freezing avoidance), and survival despite partial freezing (freezing tolerance). However, there is limited information on cold hardiness in snails. In intertidal species, freezing tolerance is the major strategy, and numerous examples of snails exhibiting seasonal fluctuations in freezing tolerance have been reported (Loomis, 1985; Ansart & Vernon, 2003). In land snails, several species have been shown to exhibit freezing avoidance by lowering their supercooling points in winter (Riddle & Miller, 1988; Ansart & Vernon, 2003). In freshwater snails, for which little information is available, the pulmonate *Gyraulus acronicus* exhibits seasonal fluctuation in cold hardiness, developing a capability for freezing tolerance during winter (Olsson, 1984).

As an invasive freshwater snail, *P. canaliculata* has adopted various strategies for overwintering in paddy fields in temperate Japan. The snails bury themselves in the paddy soil or move under rice straw before the onset of winter. This behavior is apparently to avoid cold weather and desiccation. In fact, the environment under straw was much milder, with higher humidity, than that on the soil surface, and that in the soil itself would be expected to be more favorable for overwintering than that under straw. In fact, snails that successfully buried themselves in the soil attained higher survivorship. In addition to this behavioral avoidance, this study clearly demonstrated physiological change in *P. canaliculata*, allowing it to develop cold hardiness in winter. This change possibly includes a decline in the supercooling temperature. However, other unknown physiological processes might also be involved, because *P. canaliculata* often died at temperatures above zero (see the mortalities after two days at 3°C in Fig. 1).

We suspect that *P. canaliculata* may not be fully adapted to its new habitat of temperate paddy fields in Japan. Many snails – about one third of those examined in this study – failed to bury themselves in the paddy soil, despite the fact that this would have ensured higher survivorship during winter. The situation is more serious if there is little straw in paddy fields (farmers often burn straw after the rice harvest), and no snails on the soil surface survived in such fields in Kumamoto (Kiyota & Sogawa, 1996). Even when the snails succeeded in burying themselves, the proportion that survived the winter was very low (7.8%) in this study. This suggests that the physiological enhancement developed by *P. canaliculata* before winter is insufficient. Undoubtedly, a cold winter imposes strong natural selection on the *P. canaliculata* population. We do not know whether the capability of developing cold hardiness before winter is an original ability of *P. canaliculata* in its native habitat, or a characteristic that has evolved after its invasion of temperate paddy fields. *Pomacea canaliculata* was introduced to many countries in tropical Asia where it does not encounter cold temperature stress. If we compare responses of the snails to temperature among populations in different regions, *P. canaliculata* may become a good model for studying the adaptive strategies of animals that have invaded a new habitat.

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SPECIES OF SCISSURELLIDAE AND ANATOMIDAE FROM SUB-ANTARCTIC AND ANTARCTIC WATERS (GASTROPODA: VETIGASTROPODA)

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ABSTRACT

A systematic revision on the shallow and abyssal species of Scissurellidae and Anatomidae occurring in Sub-Antarctic and Antarctic waters was carried out. This study is based on material from several field trips and museum collections. The shell morphologies of all the species are redescribed and illustrated with scanning electron micrographs. Additional information on the gross anatomy and the radula for seven species is given. A total of six anatomid and seven scissurellid species are recognized as valid in Sub-Antarctic and Antarctic waters, including three new species herein described. Anatomids are represented by the genera *Anatoma* and *Thieleella*; the species recognized for the area are: *Anatoma conica* (d'Orbigny, 1841), characterized by having a globular shell, sculptured with strong axial ribs; *Anatoma euglypta* (Pelseneer, 1903), has a biconical, delicately cancellate shell; *Anatoma shiraseae* Numanami & Okutani, 1990, a gradated shell with a distinct spiral ridge on the shoulder and cancellate ornamentation; *Thieleella amoena* (Thiele, 1912), is globose, strongly sculptured, and with flared lower portion of aperture; ***Thieleella weddelliana*** n. sp. is characterized by having a laterally expanded shell, with rounded whorls, deep umbilicus, and strong axial ribs; and ***Thieleella argentinae*** n. sp. shows a depressed and widely umbilicated shell, with cancellate sculpture. Scissurellids are represented by the genera *Scissurella*, *Ariella*, and *Sinezona*: *Scissurella clathrata* Strebel, 1908, is characterized by having an inflated shell with densely cancellate sculpture; *Scissurella petermannensis* Lamy, 1910, is sculptured with strong axial ribs; *Scissurella obliqua* Watson, 1886, is nearly smooth and has a shallow umbilicus; ***Scissurella malvinarum*** n. sp. also has a smooth shell surface, but the umbilicus is deep and surrounded by 2–6 spiral cords; in *Scissurella supraplicata* Smith, 1875, the shell is inflated and teleoconch I exhibits strong axial ribs; *Ariella subantarctica* (Hedley, 1916), has a depressed shell, sculptured with axial ribs and spiral cords, a prominent umbilical callus, and closed foramen; and *Sinezona mouchezi* (Vélain, 1876), is inflated, sculptured with strong axial ribs, showing a wide umbilicus and a large foramen. The other three scissurellid species previously described from the area – *Scissurella eucharista* Melvill & Standen, 1912; *Scissurella timora* Melvill & Standen, 1912; and *Scissurella medioplicata* Thiele, 1925 – are considered to be synonyms, and *Scissurella dalli* Bartsch, 1903, is considered a *nomen dubium*. As part of this revision lectotypes are designated for *Thielella amoena*, *Scissurella timora*, *Scissurella medioplicata*, *Scissurella obliqua*, and *Sinezona mouchezi*.

Key words: *Anatoma*, *Thieleella*, *Scissurella*, *Ariella*, *Sinezona*, Southern Ocean.

INTRODUCTION

In the last years several workers have studied the Scissurellidae and Anatomidae from the southern part of the southern hemisphere: Herbert (1986: South African species), Jansen (1999) and Geiger & Jansen (2004a, b: Australian species), Marshall (2002: New Zealand species). However, none of these studies included the species from the Sub-Antarctic or Antarctic waters, where these groups remain mainly known on the basis of rather poor descriptions that lack much necessary detail.

A total of 15 nominal species of scissurellids and anatomids are currently known from Sub-Antarctic and Antarctic waters. *Anatoma conica*

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(d'Orbigny, 1841), from the Malvinas (Falkland) Islands, was the first species described from the area. During the XIX century, *Scissurella supraplicata* Smith, 1875, from Kerguelen Islands; *Sinezona mouchezi* (Vélain, 1877) from St. Paul Island; and *Scissurella obliqua* Watson, 1886, from Kerguelen Islands were described. In the first quarter of the XX century, nine additional scissurellid and anatomid species were added: *Scissurella dalli* Bartsch, 1903, from San Jorge Gulf (Argentina); *Anatoma euglypta* (Pelseneer, 1903) from the west side of the Antarctic Peninsula; *Scissurella clathrata* Strebel, 1908, from Isla de los Estados (Argentina); *Scissurella petermannensis* Lamy, 1910, from South Shetland Islands; *Scissurella eucharista* Melvill & Standen, 1912, from Burdwood Bank (Malvinas Islands); *Scissurella timora* Melvill & Standen, 1912, from South Orkney Islands; *Thieleella amoena* (Thiele, 1912) from Gauss Station (East Antarctica); *Ariella subantarctica* (Hedley, 1916) from Macquarie Island; and *Scissurella medioplicata* Thiele, 1925, from Kerguelen Islands. After a hiatus of 65 years, *Anatoma shiraseae* Numanami & Okutani, 1990, from Günnerus Bank (East Antarctica), and *Scissurella georgica* Davolos & Moolenbeek, 2005, from South Georgia Islands were added.

With the exceptions of *Anatoma shiraseae* and *Scissurella georgica*, all the remaining Sub-Antarctic and Antarctic species of scissurellids and anatomids were described prior to the invention of the scanning electron microscope (SEM). Consequently, relevant details on shell microsculpture are insufficiently known. This fact resulted in the placement of all species in *Scissurella*; the only exceptions were *Sinezona mouchezi* and *Ariella subantarctica*, which were originally described in *Schismope*. In addition, most of these species were described on the basis of a few specimens, if not from a single one, and subsequent reports of these species are not common in the specific literature of the Southern Ocean (e.g., Hain, 1990; Dell, 1990; Linse, 1997, 1999, 2002). All these facts make it difficult to determine the actual diversity of the group in the area.

We reassess here the species diversity of Scissurellidae and Anatomidae from Sub-Antarctic and Antarctic waters, by means of redescribing and properly figuring the species currently known for the area. All descriptions are based on SEM studies, including the examination of all traceable type material. In addition, the generic placement of the species and their geographic distribution are re-examined.

MATERIAL AND METHODS

This study is based on the scissurellids and anatomids collected during several field trips performed in the southern tip of South America at both Atlantic and Pacific coasts, and throughout the Scotia Arc Islands. Additional information come from the following museum collections: Australian Museum Sydney, Australia (AMS); Koninklijk Belgisch Instituut voor Natuurwetenschappen, Brussels, Belgium (KBIN); Los Angeles County Museum, Los Angeles, California, USA (LACM); Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN); Museo de La Plata, Argentina (MLP); Muséum Nationale d'Histoire Naturelle, Paris, France (MNHN); Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand (NMNZ); National Museum of Scotland, Edinburgh, Great Britain (NMSZ); National Museums and Galleries of Wales (NMW); National Science Museum Tokyo, Japan (NSMT); The Natural History Museum, London, Great Britain (BMNH); Swedish Museum of Natural History, Stockholm, Sweden (SMNH); United States National Museum, Smithsonian Institution, Washington, D.C., USA (USNM); Zoologisches Institut und Zoologisches Museum der Universität Hamburg, Germany (ZMH); Zoologisches Museum, Berlin, Germany (ZMB); Zoologische Staatssammlung München, Germany (ZSM).

Descriptions of the shell (including protoconch) and the radula are based on SEM images. SEM images were captured on various instruments (LEO 435VP, Hitachi 3000N, Zeiss EVO 40XVP). Non-type specimens were coated with gold or gold-palladium and imaged at 10–20 kV, 100–200 pA probe current, and 10 ± 1 mm working distance in high vacuum using the secondary electron detector; instrumentation permitting (LEO, Zeiss), selected quadrants of a four quadrant backscatter detector were mixed with the secondary electron signal at approximately 10–25% to brighten up shadows. In some instances, better images of coated specimens were obtained in low variable pressure environment (30 Pa chamber pressure) at 20 kV, probe current of approximately 100 pA, and 10 ± 1 mm working distance using a Variable Pressure Secondary Electron detector (VPSE: Carl Zeiss, Cambridge). Type specimens were imaged uncoated at 40–70 Pa chamber pressure using the VPSE detector on the Zeiss EVO40 XVP.

Teleoconch I (T1) is the portion of the shell from the end of the protoconch to the onset of the selenizone. Teleoconch II (T2) is the portion of the shell from the onset of the selenizone to the apertural lip.

The indication of size is relative within each of these two families of microgastropods. For instance, Scissurellidae smaller than 1 mm are considered "small", those of 1–2 mm are of "medium size", whereas species larger than 3 mm are considered "large".

The descriptions of the external anatomy are based on gross dissection under stereoscopic microscopy. Additional information on the anatomy of the species comes from hexamethyldisilazane prepared soft parts for SEM. In the anatomical descriptions, "hindgut" is employed to refer to the last portion of the intestine, located below the roof of the pallial cavity, for which the specimens were oriented in frontal view.

Histology of select material was also performed. Either 5 μm serial sections of paraffin embedded material were stained with hematoxylin-eosin. Alternatively, specimens were embedded in JB-4 (Polysciences) medium at 0.5 bars, cut serially at 2 μm with freshly broken glass knives on a Sorval II ultramicrotome, and were stained with either multiple stain (Polysciences) or a 50%–50% mixture of 1% toluidine blue and 1% methylene blue (= Richardson's stain). Sections were inspected on a Zeiss Axioskop 2 plus with planapochromatic objectives; images were captured with a Zeiss Axiocam HRc digital camera and captured with Zeiss Axiovision LE software (Carl Zeiss, Oberkochern).

The holotype of *Anatoma josephinae* (housed at the Natural History Museum, Gotenburg, Sweden) was examined using a Zeiss DV12 stereomicroscope and a Zeiss Axiocam HRc. The protoconch was inspected under a compound microscope and measured using calibrated Carl Zeiss Axiovision LE software.

In the present paper, Scissurellidae and Anatomidae are used at family-level following Geiger & Jansen (2004a, b) and Geiger & Thacker (2005, 2006), instead as two subfamilies within Scissurellidae as previously frequently considered (reviewed in Geiger, 2003). The family and genus-level names have been treated elsewhere more extensively with respect to diagnostic characters, taxonomic history, and synonymy (Geiger, 2003).

The synonymy listings presented here mainly indicate the application of the name in the literature. However, many of these works were not illustrated, or the available illustrations are insufficient to allow positive confirmation of the correct attribution of a name to the specimen(s).

Under "Distribution" the geographic area and the bathymetric range from which the species is known is indicated; these data are based on the compilation of previous literature and the new records provided herein. Latitude and longitude inferred with secondary sources from locality names are given in square brackets; approximate coordinates (identified with the ~-symbol) are provided for imprecise locality names.

Abbreviations used: M: monotypy; OD: original designation; sh(s): empty shell(s); sp(s): life collected specimen(s); L. Latin; G. Greek; stn: station.

SYSTEMATICS

Anatomidae McLean, 1989

Diagnosis

Shell 1–11 mm, trochoid biconical, protoconch with smooth, flocculant or reticulate sculpture; slit at periphery, usually open (closed in *Sasakiconcha elegantissima* Geiger, 2006); radula with lateral tooth 5 enlarged by elongation. More comprehensive diagnoses and discussions have been provided elsewhere (Geiger 2003, 2006a, b; Geiger & Jansen 2004a, b).

Remarks

Scissurellidae have the slit or foramen above the periphery and lateral tooth 5 is enlarged by broadening.

Anatoma Woodward, 1859

Type Species (M): *Scissurella crispata* Fleming, 1828: 385, pl. 6, fig. 3.

Diagnosis

Shell 1–11 mm, trochoid biconical, protoconch with smooth or flocculant sculpture; slit at periphery of whorl, open. Flocculant sculpture characterized by small islands of shell material arranged in an overdispersed pattern; although the islands may occasionally touch one another, adjacent islands do not enclose a discrete area, and the width of the islands along their axis varies by a factor of at least three. More comprehensive diagnoses and discussions have been provided elsewhere (Geiger 2003, 2006b; Geiger & Jansen 2004a).

Anatoma conica (d'Orbigny, 1841)
Figures 1–15

Scissurella conica d'Orbigny, 1841: 470, pl. 78, figs. 7–9; Gray, 1854: 50; Munier-Chalmas, 1865: 396; Paetel, 1888: 289; Rochebrune & Mabille, 1889: H75; Pilsbry, 1890: 53, pl. 57, figs. 8–9; Carcelles, 1950: 49; Ageitos de Castellanos & Landoni, 1988: 6–7, pl. 1 fig. 4.

Anatomus conicus: Adams H. & A. Adams, 1854: 439.

Scissurella supraplicata Smith: Melvill & Standen, 1912: 345 [*non* Smith, 1875].

Schizotrochus conica [*sic*]: Carcelles & Williamson, 1951: 251.

Schizotrochus supraplicata: Carcelles & Williamson, 1951: 251 [*non* Smith, 1875].

Schizotrochus supraplicatus: Carcelles, 1953: 162 [*non* Smith, 1875].

Anatoma conica: Lozouet, 1986: 113; Dell, 1990: 76, fig. 128; Linse, 1999: 400.

Scissurella supraplicata: Ageitos de Castellanos & Landoni, 1988: 8 [*non* Smith, 1875].

Anatoma s.l. conica: Geiger, 2003: 74.

Type Locality

Malvinas (Falkland) Islands [51°30'S, 59°00'W].

Material Examined

Pacific sector of Magellan Region: 46°35'S, 75°30'W, Bahía San Andrés, N. of Golfo de Peñas, Chile, 1 m (1 sh, LACM 73-73). Strait of Magellan: 53°28'48"S, 70°21'54"W, Paso Ancho, 92 m (3 sps & 5 shs, ZMH 19588); 53°39'S, 72°15'W, Isla Carlos III, 55 m (1 sh, MLP 12060). Drake Passage: 56°19'30"S, 67°09'45"W, 121 m (1 sp, NMSZ 42838). Atlantic sector of Magellan Region: 54°52'S, 65°50'W, Ensenada Patagones, 144 m (1 sh, USNM 845403; 2 shs, LACM 71-305); 55°47'S, 66°17'W, 115 m (4 shs, NMNZ M.242839); Isla de los Estados: 54°54'S, 63°56'W, Punta

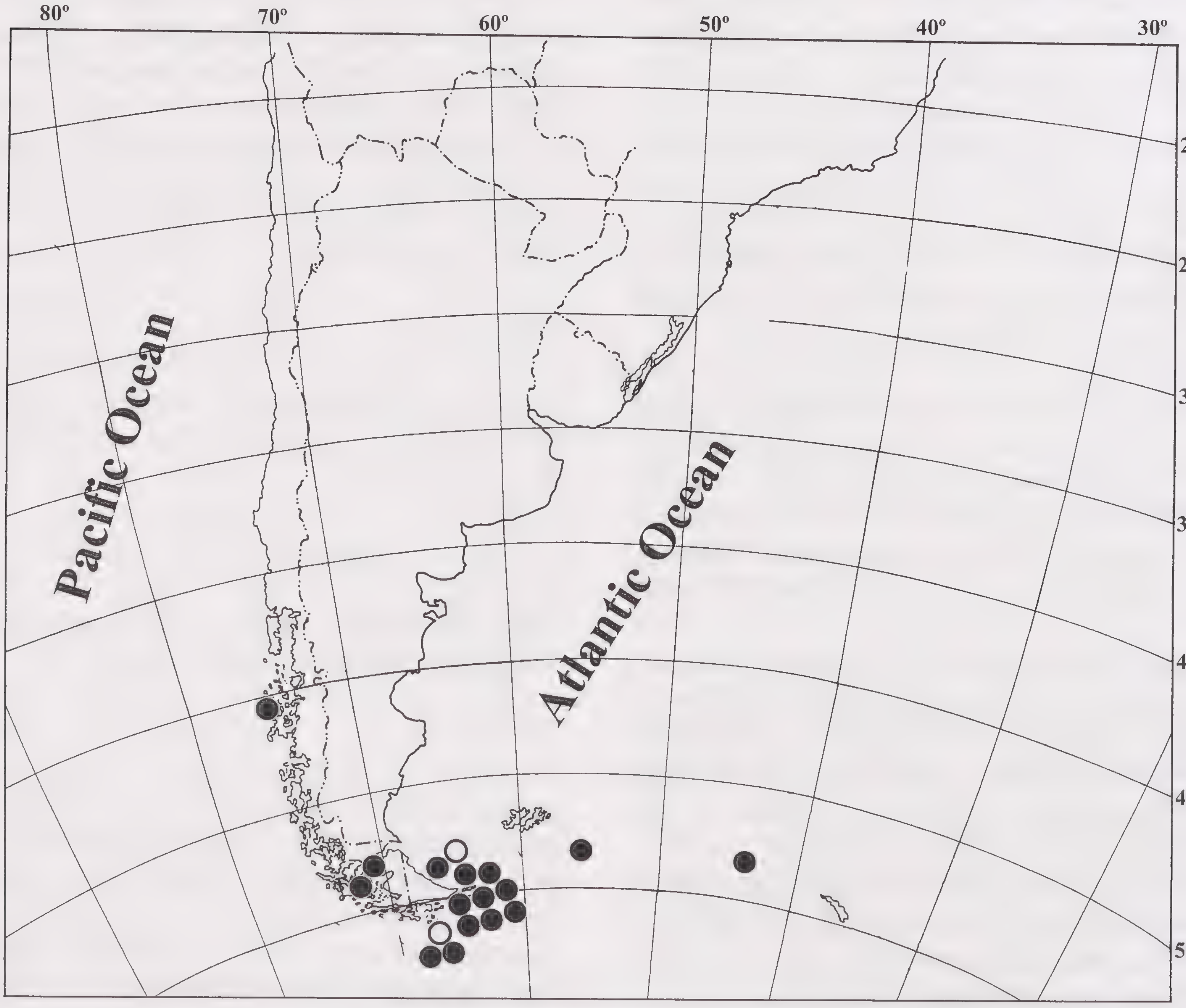


FIG. 1. Distribution of *Anatoma conica*. ●: material examined; O: bibliographic records.

Ventana, 771–903 m (1 sh, USNM 845405; 2 shs, LACM 71-329); 54°50'S, 64°29'W, Cabo San Antonio, 67–71 m (1 sh, USNM 845400); 54°34'S, 64°30'W, Cabo San Antonio, 73–76 m (4 shs, USNM 845402; 5 shs, LACM 71-352); 54°29'S, 64°40'W, Bahía Crossley, 116–20 m (1 sh, USNM 845404); 55°S, 64°48'W, Cabo San Bartolomé, 438–548 m (1 sh, LACM 71-342); 54°29'S, 64°20'W, Cabo Colnett, 109 m (3 shs, LACM 71-314); 54°50'S, 64°10'W, Cabo Kendall, 76 m (1 sh, LACM 71-334). Burdwood Bank: 54°25'S, 57°32'W, 102 m (3 shs, NMSZ 1921.143.597). Shag Rocks: 53°23'S, 42°42'W, 313 m (1 sh, MLP 12061).

Other Literature Records

54°56'S, 65°03'W, E of Tierra del Fuego, 229–265 m; 55°47'S, 66°17'W, off Cape Horn, 115 m (Dell, 1990).

Distribution

Magellan Region, extending eastwards to Shag Rocks. 1–548 m (living specimens rather at 92–121 m) (Fig. 1).

Etymology

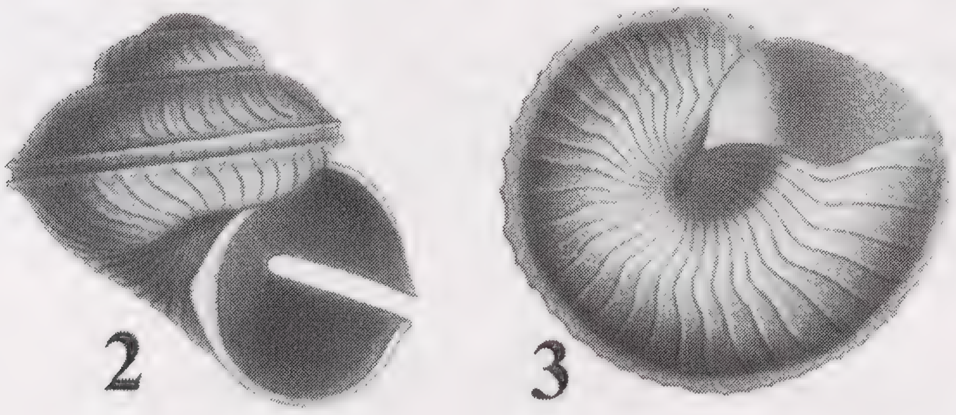
Conica: (L.) cone-shaped.

Diagnosis

Shell globular, sculptured with numerous strong axial ribs, fine spiral threads on teleoconch II; protoconch with flocculant ornamentation.

Description

Shell trochiform, globular, inflated, medium size (up to 1.8 mm in diameter), suture approximately one width of selenizone below selenizone (Figs. 2, 4–6). Protoconch of approximately one whorl, 160–205 μm in diameter, with flocculant sculpture; margin of protoconch convex, without apertural varix (Figs. 13–15). Teleoconch I of 1/2 to 2/3 whorls, with single spiral cord in position of selenizone on early teleoconch I, disappearing close to onset of selenizone; 10–21, occasionally bifurcated, axial ribs (Figs. 10–12). Teleoconch II of up to two rounded whorls, with convex shoulder. Shell surface cancellate: axial sculpture of strong ribs, 26 on shoulder of first whorl, 31–34 on last whorl (occasionally bifurcating near selenizone); approximately 60 on base; spiral threads weak, beginning at start of selenizone, gradually increasing in number with growth, approximately 18–20 at apertural margin, 25 on base (Figs. 4–12). Selenizone at periphery, relatively wide, sculptured with low axial ribs, surrounded by narrow keels (Figs. 4–6). Umbilicus open, wide, deep; walls with continuous transition to base (Figs. 3, 7–9); funiculus present. Aperture rounded. Animal unknown.



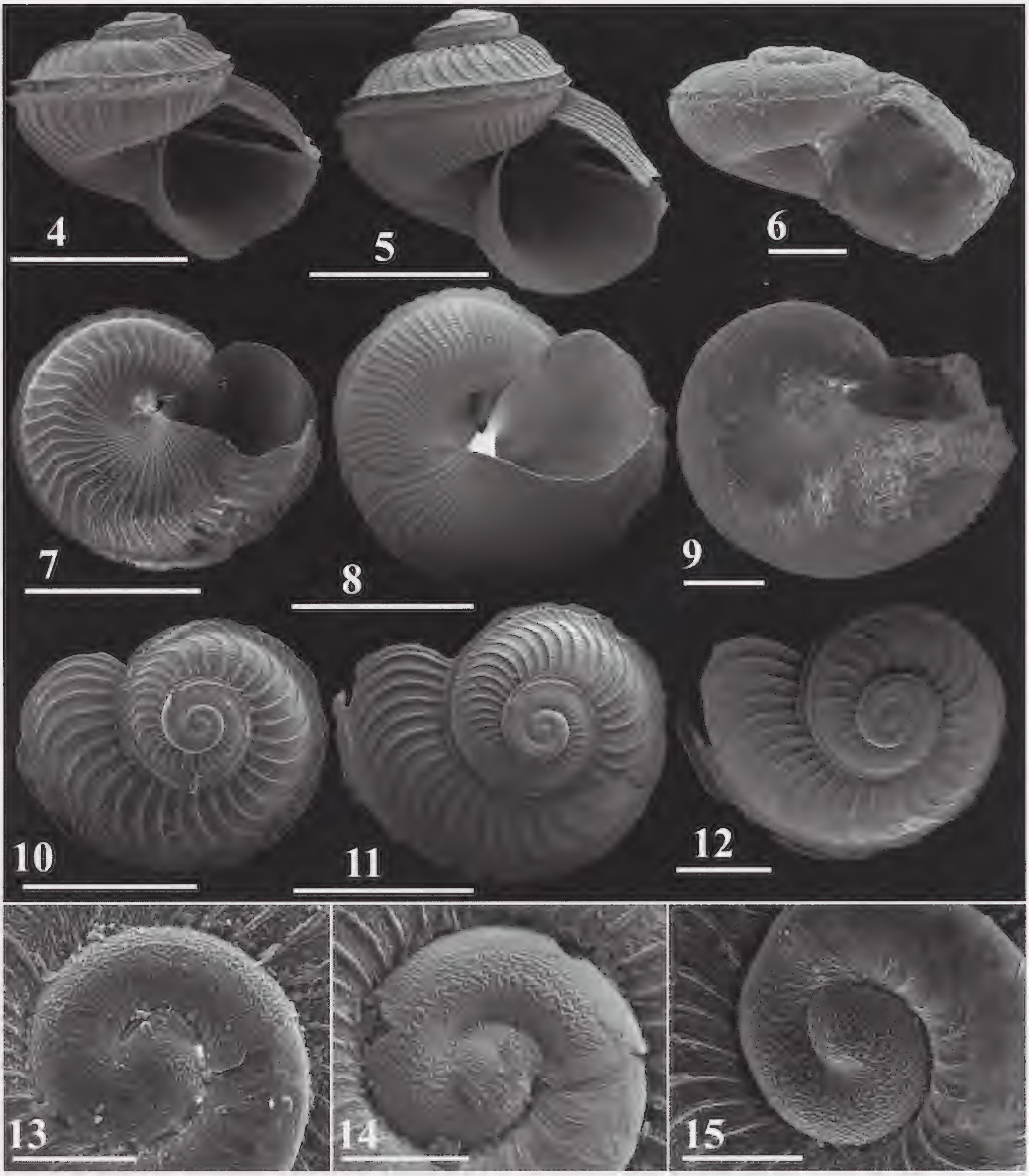
FIGS. 2–3. *Anatoma conica*. Original figures by d'Orbigny (1841). FIG. 2: Apertural view; FIG. 3: Umbilical view.

Remarks

The type material of *Anatoma conica* is probably lost; it was originally deposited in the BMNH (Gray, 1854), but not found currently there (Geiger, pers. obs.; K. Way, pers. comm.). However, we consider the original description and illustrations (Figs. 2–3) to be adequate to recognize this species; in addition, the topotypic specimens examined during this study allowed us to confirm the identity of this taxon.

Anatoma conica is most similar to the Antarctic *Thieleella amoena* (Thiele, 1912), from which it differs by a wider shoulder, stronger axial ribs, and flocculent (instead of reticulate) protoconch ornamentation. Other similar species are *Thieleella gunteri* (Cotton & Godfrey, 1933) from southern Australia (Geiger & Jansen, 2004a) and *Thieleella flemingi* Marshall, 2002, from New Zealand, which also show reticulate protoconch sculpture. *Anatoma conica* can be easily separated from the Antarctic *Anatoma euglypta* (Pelseneer, 1903) by its more globular shell, as opposed the overall biconical shape of *A. euglypta*. In addition, *Anatoma conica* has fewer but more elevated axial ribs, particularly on the shoulder, while the axials are more numerous and the strength of the sculpture is more homogeneous on shoulder and base in *A. euglypta*.

The specimens from Burdwood Bank, previously referred by Melvill & Standen (1912) to *Scissurella supraplicata* (NMSZ 1921.143.596) are here re-identified as *Anatoma conica*.



FIGS. 4–15. *Anatoma conica*. FIGS. 4, 7, 10, 13: Specimen from 55°47'S, 66°17'W, Estrecho de Magallanes, 115 m (NMNZ M.42839); FIGS. 5, 8, 11, 14: Specimen from 56°19'S, 67°09'W, Drake Passage, 121 m (BMNH 20000096); FIGS. 6, 9: Specimen from 54°34'S, 64°30'W, Cabo San Antonio, Isla de los Estados, 73–76 m (USNM 845402); FIGS. 12, 15: Specimen from 53°23'S, 42°42'W, Shag Rocks, 313 m (MLP 12061); FIGS. 4–6: Apertural view; FIGS. 7–9: Umbilical view; FIGS. 10–12: Apical view; FIGS. 13–15: Protoconch. Scale bars FIGS. 4–5, 7–8, 10–11 = 1 mm; FIGS. 6, 9 = 200 µm; FIG. 12 = 500 µm; FIGS. 13–15 = 100 µm.

Anatoma euglypta (Pelseneer, 1903)
Figures 16–50

Scissurella crispata Fleming: Watson, 1886: 113 [*non* Fleming; in part., specimens from stn 145, R/V Challenger is most likely *Anatoma euglypta*; see Gaillard (1971) for discussion].

Scissurella euglypta Pelseneer, 1903: 17–18, pl. 4, fig. 43; Thiele, 1912a: 187; Thiele, 1912b: 8, pl. 1, figs. 1, 2, 16; Melvill & Standen, 1912: 345; Hedley, 1916a: 85.

Schizotrochus euglyptus: Powell, 1951: 79; Carcelles, 1953: 162; Powell, 1958: 169, 179; Powell, 1960: 126; Arnaud, 1972a: 113; Arnaud, 1974: 641; Egorova, 1982: 11, fig. 72; Cantera & Arnaud, 1985: 32; Castellanos et al., 1987: 58, pl. 1, fig. 1; Ageitos de Castellanos & Landoni, 1988: 9–10, pl. 1, fig. 7.

Anatoma euglyptus [*sic*]: Hain, 1990: 33–34, pl. 1, fig. 1; pl. 28, fig. 7; Numanami & Okutani, 1990: 94–100, figs. 2–5; Numanami & Okutani, 1992: 172.

Anatoma euglypta: Dell, 1990: 75, fig. 129; Numanami, 1996: 12–18, figs. 6A–D; Geiger, 2003: 7; Geiger & Thacker, 2005: 49, fig. 1 [phylogeny].

Hainella euglypta: Bandel, 1998: 37, pl. 12, figs. 5–7.

Type Localities

70°15'S, 84°06'W, 569 m (stn 780, R/V Belgica), 71°19'S, 87°37'W, 400 m (stn 998, R/V Belgica), 70°20'S, 83°23'W, 459 m (stn 1030, R/V Belgica) (OD).

Material Examined

Photographs of one of the syntypes (KBIN). Atlantic sector of the Magellan Region: Isla de los Estados: 54°50'S, 63°50'W, off Punta Fallows, 342–353 m (1 sp, LACM 71-331); 54°55'S, 63°56'W, Punta Ventana, 771–903 m (2 shs, LACM 71-329; 2 shs, USNM 845406). Drake Passage: 56°19'S, 67°09'W,

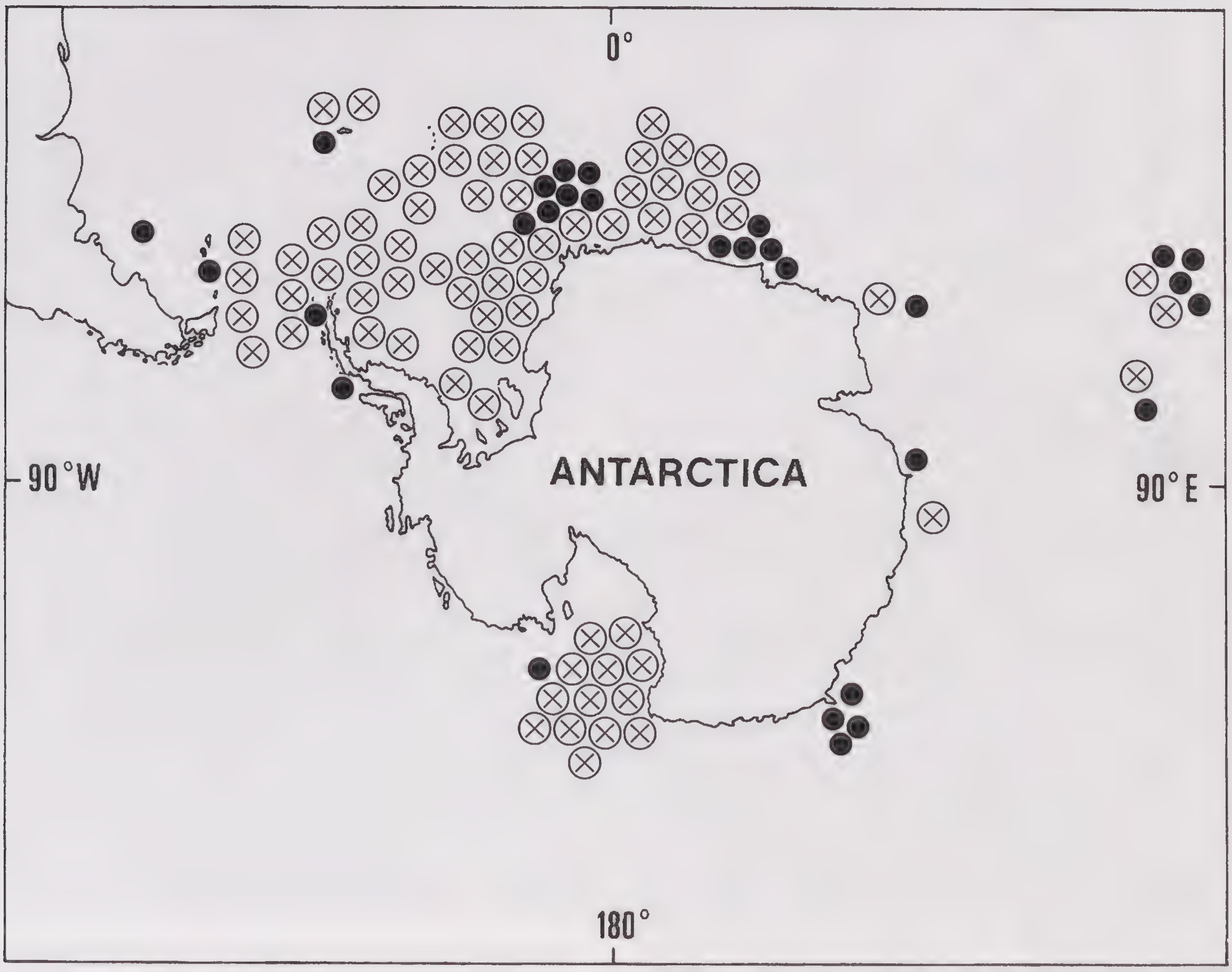
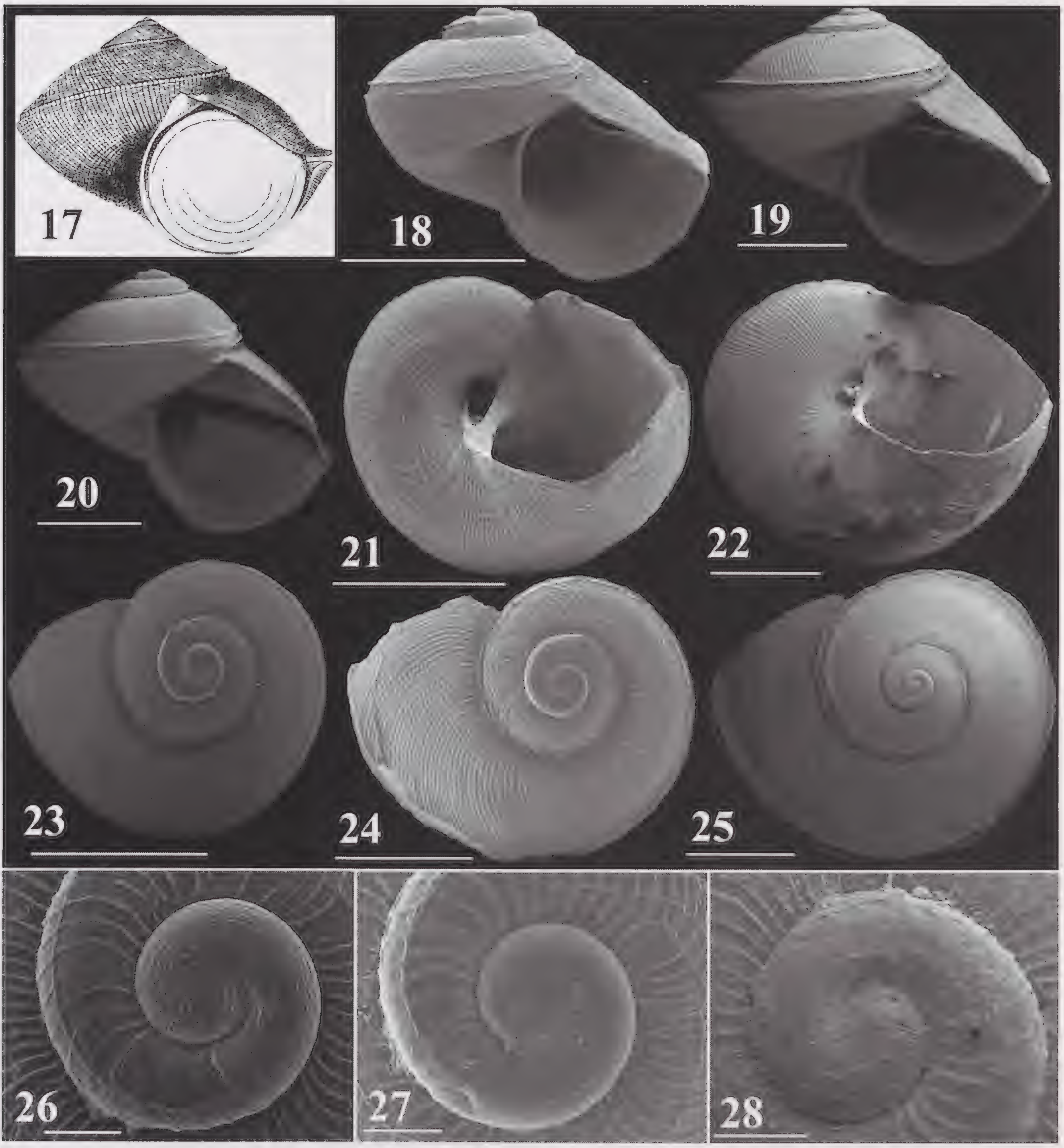


FIG. 16. Distribution of *Anatoma euglypta*. ⊗: material examined; ●: bibliographic records.

121 m (12 sps, BMNH 20000096). Burdwood Bank: 54°30'S, 56°08'W, 286–290 m (8 sps & 5 shs, MLP 120602; 3 sps, ZSM Mol 20021528; 5 sps & 1 sh, ZSM Mol 20021278); 54°25'S, 57°32'W, 102 m (1 sh, NMSZ 1921.143.596). South Georgia: 54°30'S, 35°50'W, 94 m (3 sps, MLP 7281; 4 sps, MACN-In 36273); 53°58'S, 37°09'W, 138 m (2 sps, MLP 7301; 1 sp, MACN-In 36274). South Sandwich Islands: 57°40'S, 26°25'W, 309 m (76 sps & 15 shs, MLP 12063; 9 sps & 2 shs, ZSM Mol 20021094; 20 sps & 3 shs, ZSM Mol 20021351); 57°40'S, 26°24'W, 263–270 m (14 sps & 6 shs, MLP 12064; 4 sps, ZSM Mol 20021459); 57°40'S, 26°27'W, 587 m (17 sps & 3 shs, MLP 12065; 5 sps, ZSM Mol 20021491; 50 sps, ZSM Mol 20021328); 59°54'S, 32°28'W, 521 m (7 sps & 2 shs, MLP 12066; 1 sh, ZSM Mol 20021449); 59°42'S, 27°57'W, 332 m (1 sp, ZSM Mol 20021488); 58°24'S, 25°01'W, 2276 m (2 sps, ZSM Mol 20021126); 58°16'S, 24°53'W, 2940–2945 m (1 sp, ZSM Mol 20021580; 1 sp, ZSM Mol 20021142); 58°25'S, 25°01'W, 2285–2310 m (2 sps, ZSM Mol 20021123); 58°44'S, 25°10'W, 774–756 m (5 sps, ZSM Mol 20041498; 4 sps, ZSM Mol 20041496); 58°25'S, 25°01'W, 2313–2281 m (1 sh, ZSM Mol 20041499). Discovery Bank: 60°08'S, 34°56'W, 374 m (2 sp, MLP 12067; 1 sp, ZSM Mol 20021076). South Shetland Island: 62°40'S, 54°45'W, 210–220 m (3 shs, NMNZ M.242836); 60°39'S, 53°57'W, 2889–2893 m (1 sp, ZSM Mol 20021203; 1 sp, ZSM Mol 20021215); 63°01'S, 61°09'W, 311–365 m (1 sp, ZSM Mol 20010184); 63°26'S, 62°15'W, 119–124 m (1 sp, NMNZ M.242837). Crozet Island: NE of Isle de la Possession, 22 m (2 shs, MNHN); 46°23'S, 51°50'E, 22 m (1 sh, MNHN). Kerguelen Islands: 46°48'S, 70°30'E, 1218 m (1 sh, MNHN). Antarctic Peninsula: 62°55'S, 57°39'W, 214–218 m (1 sh, ZSM Mol 20012475; 1 sh, ZSM Mol 20012470); 63°04'S, 57°31'W, 94–95 m (1 sh, ZSM Mol 20012476); 62°30'S, 56°56'W, 909–911 m (1 sp, ZSM Mol 20012889); 62°55'S, 57°39'W, 214–218 m (2 sps, ZSM Mol 20012887); 63°04'S, 57°31'W, 94–95 m (1 sp, ZSM Mol 20012883); 63°04'S, 57°33'W, 93–97 m (4 sps, ZSM Mol 20012884); 63°04'S, 57°31'W, 94–95 m (5 sps, ZSM Mol 20012888). Weddell Sea: 70°57'S, 10°28'W, 250 m (1 sh, ZSM Mol 20012471); 71°07'S, 11°28'W, 112 m (10 shs, ZSM Mol 20012468); 71°09'S, 13°12'W, 765–840 m (1 sh, ZSM Mol 20012474); 71°09'S, 13°12'W, 765–840 m (1 sh, ZSM Mol 20012886); 70°48'S, 10°42'W, 500 m (1 sh, ZSM Mol 20012473); 70°40'S, 07°40'W, 100 m (1 sh, ZSM Mol 20012472); 70°49'S, 10°38'W, 316 m (1 sp, ZSM Mol 20012885); 70°50'S, 10°35'W, 251–271 m (1 sh, ZSM Mol 20010077); 65°18'S, 53°22'W, 2081–2087 m (1 sp, ZSM Mol 20021138); 71°18'S, 13°58'W, 1040–1048 m (1 sp & 1 sh, ZSM Mol 20051033; 5 sps, & 1 sh, ZSM Mol 20051016); 70°31.08'S, 14°34.83'W, 4384–4420 m (1 sp, ZSM Mol 20051091); 62°46.7'S, 53°02.5'W, 1579–1584 m (22 sps, ZSM Mol 20051106); 75°34'S, 26°43'W, 229 m (1 sh, USNM 612957); 77°37'S, 43°12'W (1 sh, USNM 612857); 77°40'S, 30°35'W, 384 m (1 sh, USNM 638831). Ross Sea: 72°05'S, 172°08'E, E of Cape Hallett, Moubray Bay, Victoria Land, 348–352 m (2 sps, USNM 904244); 72°05'S, 172°15'E, 392 m (6 shs, USNM 613010); 72°05'S, 172°08'E, 344–351 m (5 shs, NMNZ M.242835); 73°40'S, 175°17'E, Coulman Islands, 521 m (1 sh, USNM 612841); 72°03'S, 172°38'E, 342–360 m (3 shs, NMNZ M.242834); 77°S, 166°E, Scott Base, Cape Armitage, McMurdo Base (2 shs, NMNZ M.218133); 72°20'S, 174°50'E, 465–468 m (2 shs, NMNZ M.242833); 77°05'S, 177°12'E, 362 m (5 shs, NMNZ M.242831); 76°42'S, 179°44'E, 318–472 m (2 shs, NMNZ M.242832); McMurdo Sound: 76°55'S, 164°45'E, SE of Mt Larsen, (1 sh, AMS 31466); 72°08'S, 172°10'E, Cape Hallett, Burton Island, 433 m (2 shs, USNM 878219); 77°26'S, 169°39'E, 321 m (6 shs, USNM 612780). East Antarctica: 66°10'S, 49°41'E, off Enderby Land, 300 m (3 shs, AMS 376618); 66°33'S, 93°09'E, Burton Island, Davis Sea, 80 m (1 sh, USNM 878215); Lazarev Sea: 69°59'S, 08°44'E, 161–185 m (1 sh, SMNH 82903); 69°46'S, 10°01'E, 338–343 m (4 shs, SMNH 82902); 70°00'S, 03°56'E, 568–644 m (20 shs, SMNH 82900); 69°43'S, 10°46'E, 432 m (20 shs, SMNH 82897); 70°19'S, 03°16'E, 191–204 m (30 shs, SMNH 82895); 70°24'S, 06°08'E, 118–126 m (4 shs, SMNH 82896); 69°50'S, 08°00'E, 161–185 m (30 shs, SMNH 82904); 69°59'S, 08°00'E, 161–185 m (30 shs, SMNH 82901; 1 sh, SMNH 82898); 69°57'S, 05°04'E, 210–213 m (8 shs, SMNH 82899); 69°59'S, 05°08'E, 661–742 m (20 sps, SMNH 82905); 69°57'S, 06°20'E, 280–298 m (20 sps, SMNH 82906).

Other Literature Records

Off Argentina: 46°15'S, 60°09'W, 570–600 m (Castellanos et al., 1987). Burdwood Bank, 102 m (Melvill & Standen, 1912). South Georgia: 54°01'S, 36°26'W, off mouth of Stromness Harbour, 155–178 m (Powell, 1951). Antarctic Peninsula: 64°56'S, 65°35'W, Bismarck Strait, Palmer Archipelago, 93–130 m (Powell, 1951); 68°30'S, 69°W, 64 m (Dell, 1990). Weddell Sea: 70°30'S, 08°20'W, 267 m; 72°52'S, 19°37'W, 481 m; 72°28'S, 17°23'W, 300 m; 72°51'S, 19°20'W, 420 m; 72°53'S, 19°13'W, 401 m; 70°49'S, 10°45'W, 449 m; 72°58'S, 11°07'W, 347 m (Hain, 1989). East Antarctica: Gauss station (Thiele, 1912a); 65°50'S, 54°23'E, off Enderby Land, 220 m (Powell, 1958); 66°21'S, 138°28'E, off Adelie Land, 640 m (Powell, 1958); Archipel de la Pointe Géologie, between Bernard and Curie Islands, 135–140 m (Arnaud, 1972a); 66°40'S, 139°51'E, Cap Géodésie, 180–200 m (Arnaud,



FIGS. 17–28. *Anatoma euglypta*. FIG. 17: Original figure by Pelseneer (1903); FIGS. 20, 23, 26: Specimen from 72°05'S, 172°15'E, Ross Sea, 392 m (USNM 613010); FIGS. 18, 21, 24, 27: Specimen from 62°55'S, 57°39'W, Antarctic Peninsula (ZSM Mol 20012475); FIGS. 19, 22, 25, 28: Specimen from 54°50'S, 63°50'W, Isla de los Estados (LACM 71-331); FIGS. 17–20: Apertural view; FIGS. 21–22: Umbilical view; FIGS. 23–25: Apical view; FIGS. 26–28: Protoconch. Scale bars FIGS. 18–25 = 1 mm; FIGS. 26–28 = 100 µm.

1972a); 66°40'S, 139°51'E, Cap Géodésie, 230–250 m (Arnaud, 1972a); 66°40'S, 139°51'E, Cap Géodésie, 170–180 m (Arnaud, 1972a); Breid Bay: 70°10'S, 24°E, 271–310 m (Numanami & Okutani, 1992); 70°09'S, 23°46'E, 275–283 m (Numanami, 1996); 70°09'S, 24°01'E, 295–310 m (Numanami, 1996); 70°08'S, 24°16'W, 270 m (Numanami, 1996); 70°13'S, 24°25'E, 276–289 m (Numanami, 1996). Ross Sea: 72°12'S, 170°20'E, 203 m (Dell, 1990). Kerguelen Islands: 46°47'S, 70°30'E, 1218 m (Cantera & Arnaud, 1985). Crozet Islands: 46°18'S, 51°30'E, 375 m (Cantera & Arnaud, 1985); 46°21'S, 51°33'E, 190 m (Cantera & Arnaud, 1985); 46°18'S, 50°47'E, 290 m (Cantera & Arnaud, 1985); 46°32'S, 51°51'E, 195 m (Cantera & Arnaud, 1985).

Distribution

Circumantarctic, extending northwards up to off Argentina, the Scotia Arc Islands, and the Sub-Antarctic islands Kerguelen and Crozet, associated to Antarctic and Sub-Antarctic waters. 22–4420 m (living specimens: 94–4420 m) (Fig. 16).

Etymology

Eu-: (G.) true; *-glypta*: *Glypho* (G.), incise, cut in.

Diagnosis

Shell large, trochoid-biconical, laterally expanded, sculptured with fine, low, and densely cancellate threads on teleoconch II.

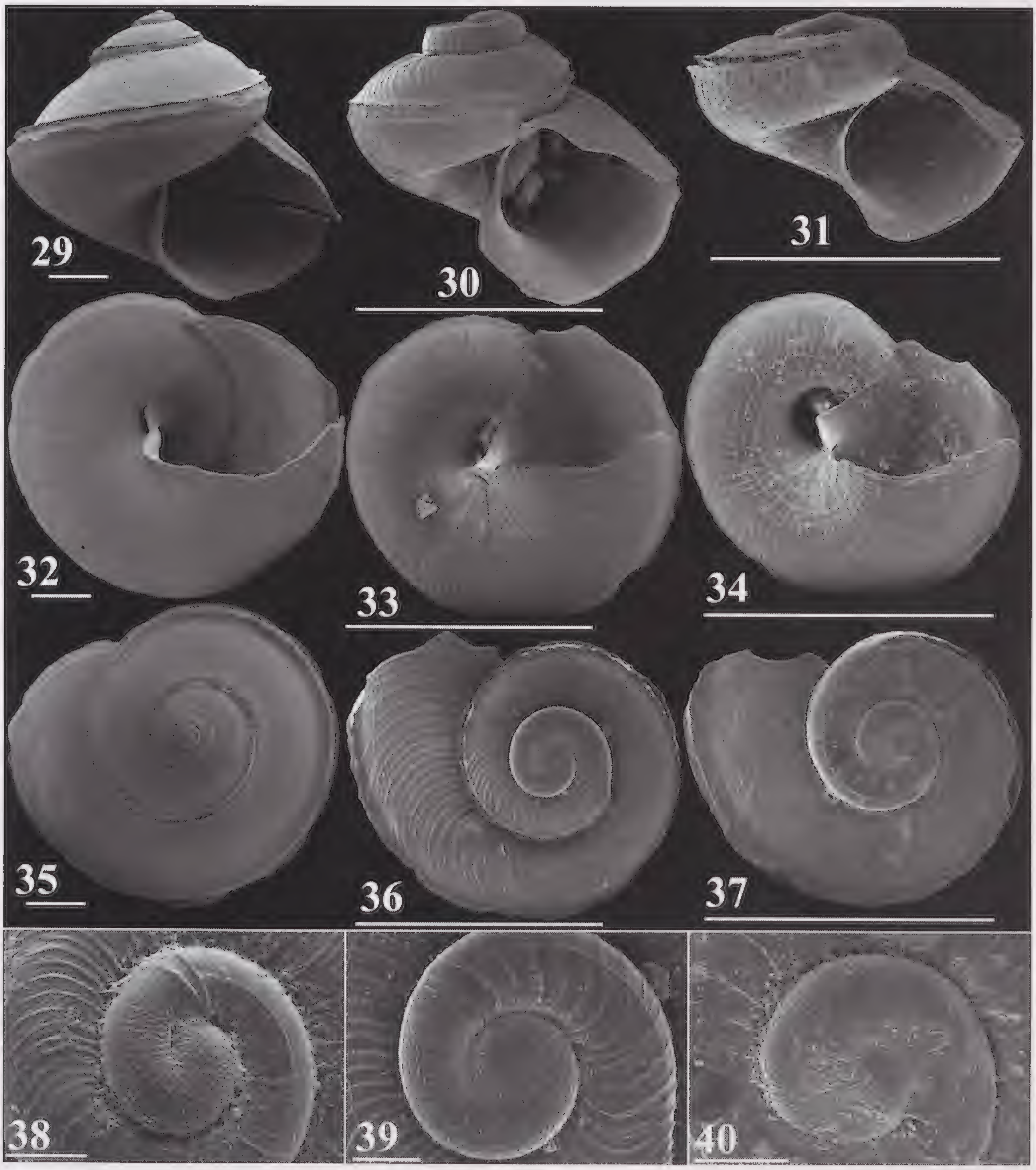
Description

Shell large (up to 11 mm in diameter), trochoid, biconical, wider than high, particularly in early juveniles (Figs. 17–19, 29–31). Protoconch of one whorl, 241–270 µm in diameter, with flocculent sculpture; apertural margin slightly sinusoid, without varix (Figs. 26–28, 38–40). Teleoconch I of 1/2 to 5/8 whorls, sculptured with 15–25 fine axial lines; interstices with fine, low growth lines; axials often increasing in strength with growth (Figs. 23–25, 35–37). Teleoconch II of up to least three whorls (in a 5.6 mm specimen: Fig. 37). Base slightly convex, with approximately 50 fine axial lines in specimens of 1 mm, approximately 150 in specimens of 5.6 mm, crossed by approximately 15 finer spiral threads at 1 mm and 50 spiral threads at 5.6 mm (Figs. 20–22, 32–34). Selenizone at periphery, with distinct axial lamellae, keels low, thin, fragile. Slit open, with margins parallel in small specimens, converging to almost touching at apertural margin in fully grown specimens (Figs. 17–19, 29–31). Umbilicus open, continuously sloping with base, wide in juveniles, progressively narrowing with growth (Figs. 20–22, 32–34). Funiculus present in specimens up to approximate 1.5 mm, not evident in larger ones. Aperture rounded. Operculum multispiral, with central nucleus, yellowish in color.

Head-Foot: Color pale cream, uniform. Cephalic tentacles long, circular, densely papillate; papillae tubular, somewhat enlarged at base, about 25–32 µm in length, arranged in verticiles (Fig. 46). Eyestalks short, fused at base of cephalic tentacles; eyes black, with lens (Figs. 42, 50). Head separated from foot by wide neck, with one pair of smooth tentacles on each side; anterior neck tentacle folliaceous, posterior neck tentacle cylindrical, sometimes bifurcated (Fig. 42). Three pairs of conical, denselly papillate, epipodial tentacles, all with one postero-basal foliated expansion (epipodial sense organ?); first epipodial tentacle divided basally forming two tentacles (Fig. 42). Papillae as in cephalic tentacles (Figs. 44–45). Foot long, narrow, with central longitudinal grove; anterior pedal mucus gland located in longitudinal groove at anterior region of foot. Columellar muscle thick, trapezoidal in outline.

Pallial Cavity: Extending over 1/4 whorl, deeply sinuate in median line, with enlarged mantle border, densely papillate internally. One papillate conical tentacle, as long as depth of mantle cavity, located at end of median sinuation; smaller, smooth tentacles surrounded it at both sides. Two large bipectinate ctenidia spanning most of dorsal mantle cavity (Figs. 41–42). Ctenidia with long, inflated basal third, distally rounded filaments; left ctenidium larger by 10%, with anterior outwards fold, provided with small indentations in margin; right ctenidium without distal fold. Osphradium not found.

Digestive System: Snout broad, tubular, rounded terminally. Jaw plate small, pteriform in outline, paved with rows of rhomboidal shingles, dark brownish in color (Fig. 47). Odontophores large, elongate, enlarged posteriorly, formed by one pair of fused cartilaginous plates (Fig. 50). Radular ribbon longer than odontophore. Esophagus as large as radula; anterior, medial esophagus walls with transversal folds; anterior, medial, posterior esophagus similar in width. Salivary glands elongate, sac-like, larger than odontophore, pinkish; salivary ducts narrow, long, opening ventrally into esophagus. Stomach half as long as esophagus. Intestine very long, twisted; hindgut forming two widely open lops, dorsally located. Anus opening into postero-central part of pallial cavity. Digestive gland extending for first 1 1/2 whorls.



FIGS. 29–40. Ontogenetical variability of *Anatoma euglypta*. FIGS. 29, 32, 35, 38: Specimen from 57°40'S, 26°25'W, South Sandwich Islands, 309 m (ZSM Mol 20021094); FIGS.: 30, 33, 36, 39: Specimen from 71°07'S, 11°28'W, Weddell Sea, 112 m (ZSM Mol 20012468); FIGS. 31, 34, 37, 40: Specimen from 70°48'S, 10°42'W, Weddell Sea, 500 m (ZSM Mol 20012473); FIGS. 29–31: Apertural view; FIGS. 32–34: Umbilical view; FIGS. 35–37: Apical view; FIGS. 38–40: Protoconch. Scale bars FIGS. 29–37 = 1 mm; FIGS. 38–40 = 100 µm.

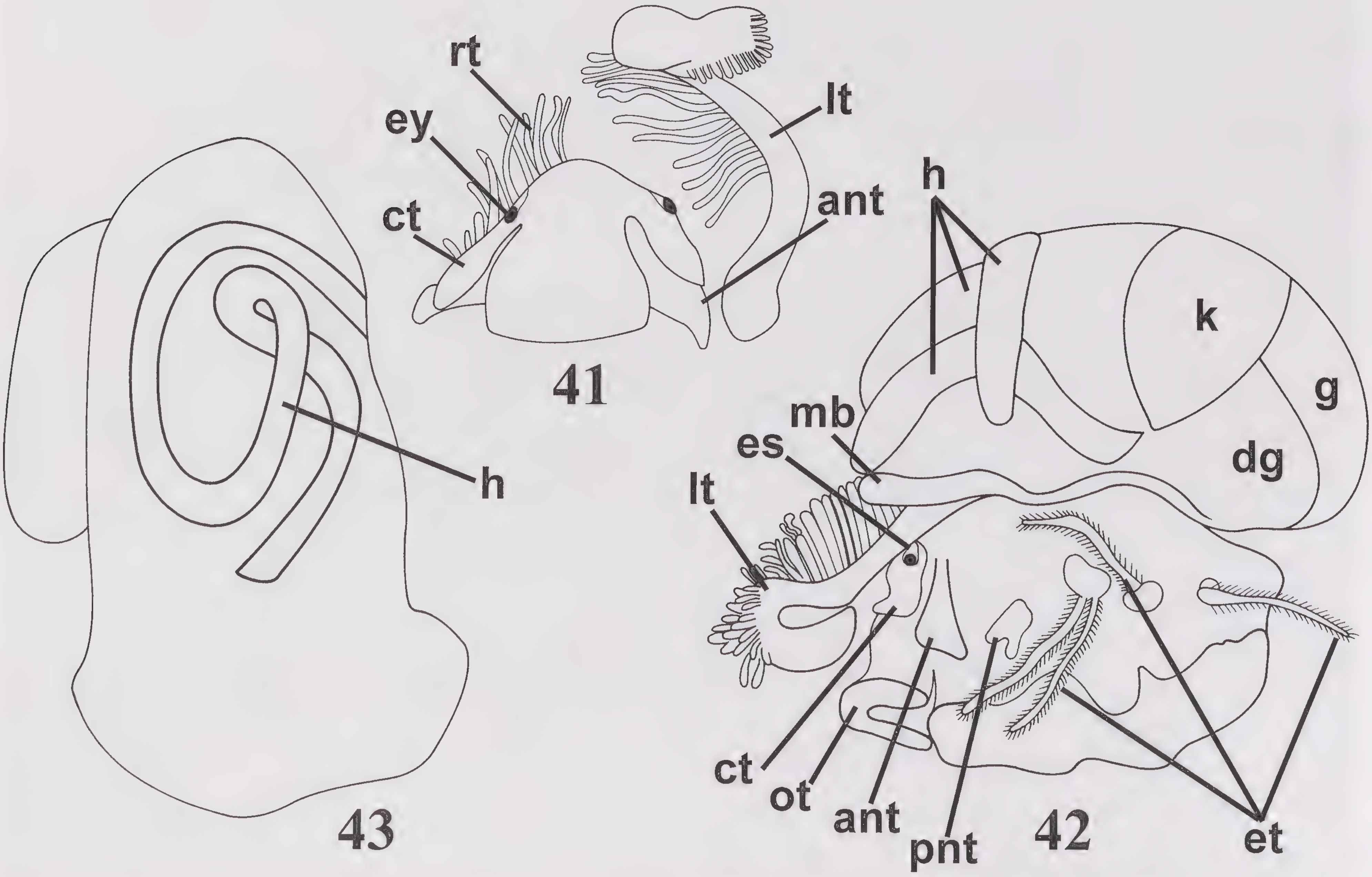
Radula (Figs. 48–49): Rhipidoglossate. Rachidian tooth trapezoidal at base, cusp with denticles parallel to radula rows; central denticle largest, six narrower pointed denticles on each side. Five pairs of lateral teeth; laterals 1–3 similar in outline, overlapping at their widely expanded bases; cusps elongate, tongue-like, with a sharp main denticle, narrower, slightly curved denticles at both sizes: 6–7 on outer side, 4–5 on inner side; fourth lateral tooth narrower than laterals 1–3, with one sharp main denticle, 3–5 additional tiny denticles on outer side; lateral tooth 5 claw-shaped, well separated at base from other laterals, with one main terminal denticle, several smaller denticles on both sides. Marginal teeth numerous (~ 30), long, narrow, becoming more delicate towards edge of radula; first marginal tooth enlarged laterally at base; shaft, cusp similar to that of remaining marginal teeth.

Nervous System: Pair of fused cerebro-pleural ganglia located on sides of buccal bulb; pedal ganglia large, located ventral to oral tube.

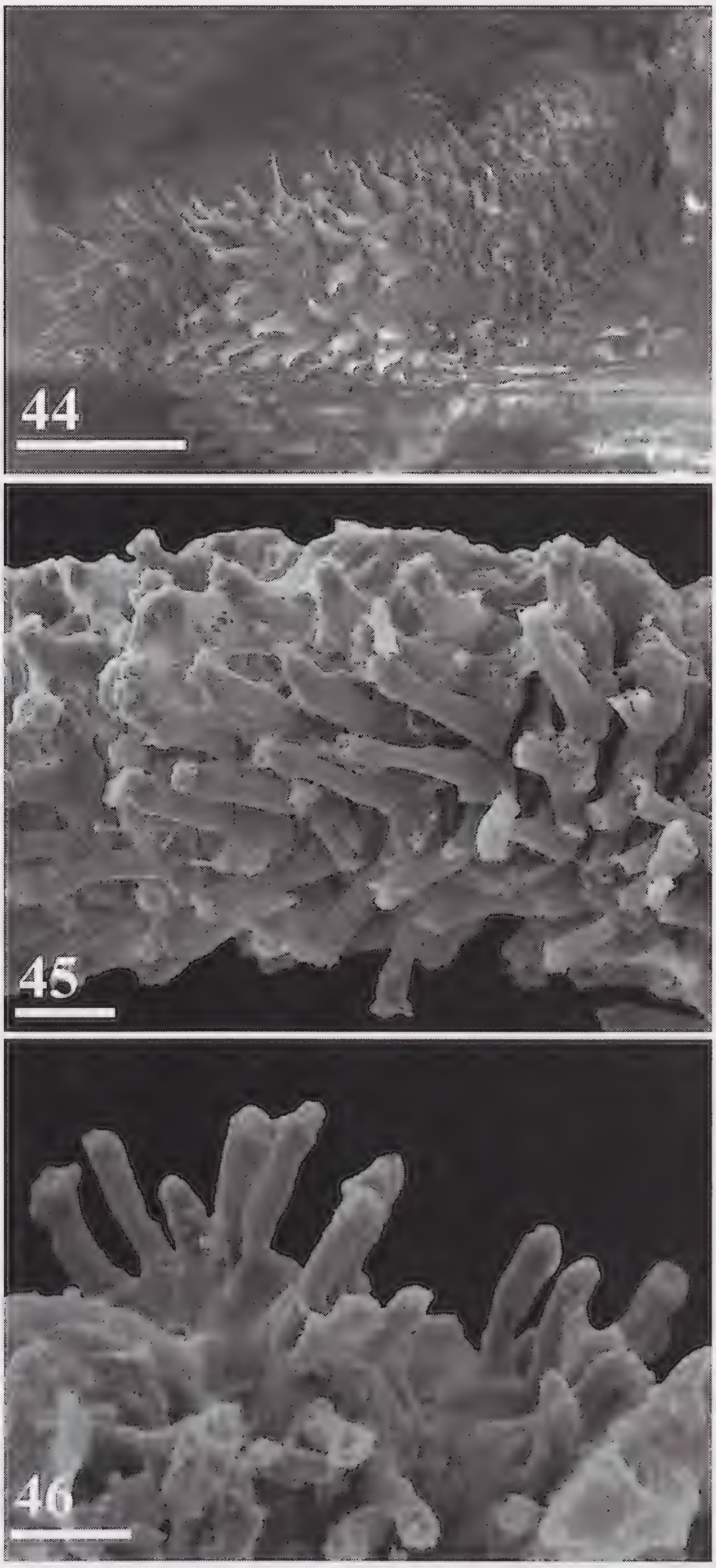
Remarks

The type material of *Anatoma euglypta* is housed in KBIN. Photographs of one specimen were examined during this study. The specimen corresponds well with the original illustration as well as with what is generally refered to as that species (see synonymy above). As we have not been able to personally examined the type material, we prefer not to designate a lectotype.

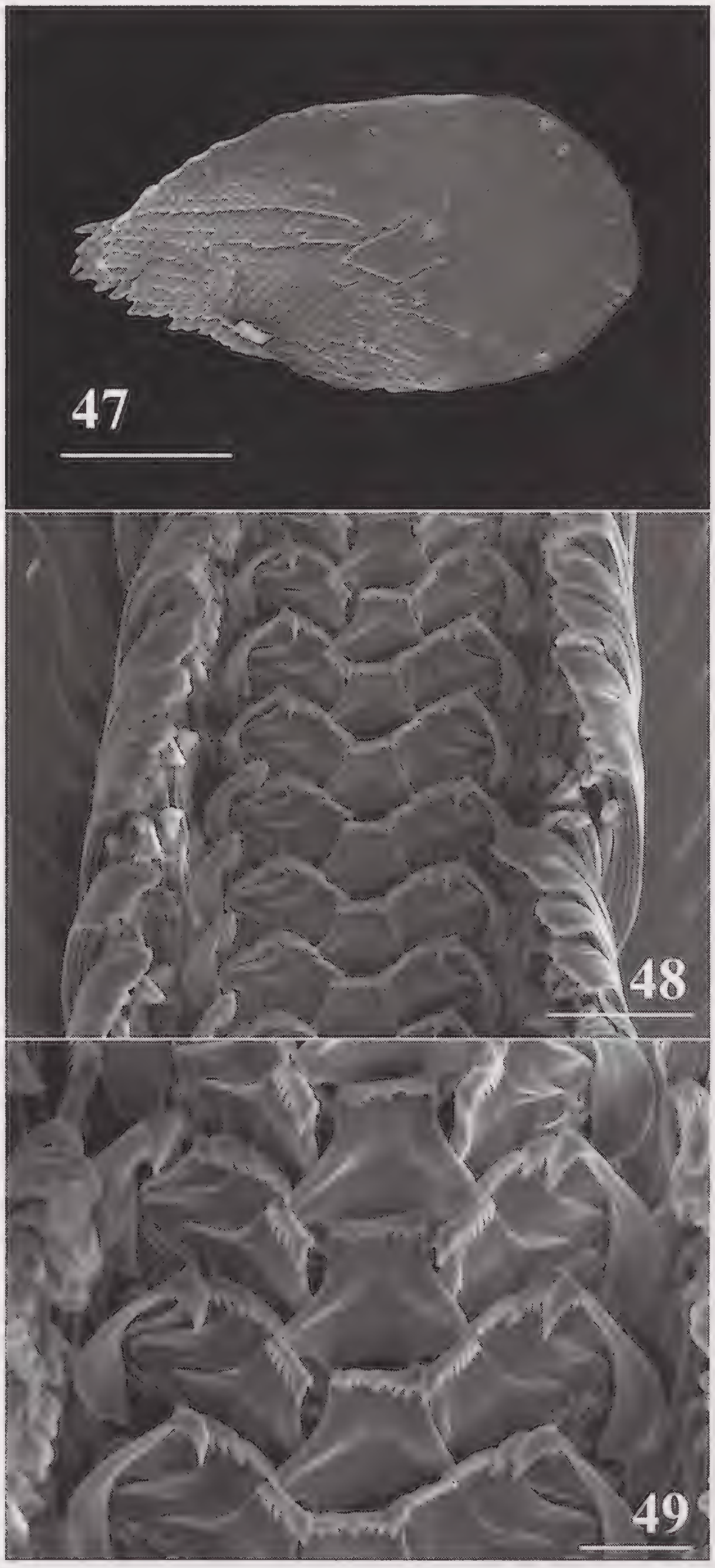
Anatoma euglypta is the largest of all anatomid species with up to 11 mm shell size. The Antarctic *Anatoma shiraseae* Numanami & Okutani, 1990, differs from *A. euglypta* in having a keel on the shoulder, a distinctive constriction below the selenizone, and coarser sculpture. *Thieleella amoena* (Thiele, 1912) has a more globose shell, which is as wide as high, a reticulate protoconch, and fewer and more prominent axial lines than *A. euglypta*. *Anatoma conica* has a globose shell outline, the shell sculpture is much stronger and less dense and the suture is deeper.



FIGS. 41–43. *Anatoma euglypta*. Anatomy. FIG. 41: Frontal view; FIG. 42: Lateral view; FIG. 43: Detail of hindgut. ant: anterior neck tentacle; ct: cephalic tentacle; dg: digestive gland; es: eye stalk; et: epipodial tentacle; ey: eye; g: gonad; i: intestine; k: kidney; lt: left ctenidium; mb: mantle border; ot: oral tube; pnt: posterior neck tentacle.



FIGS. 44–46. *Anatoma euglypta*. FIG. 44: General view of epipodial tentacle; FIG. 45: Details of epipodial tentacles; FIG. 46: Detail of cephalic tentacle. Scale bars FIG. 44 = 100 µm; FIGS. 45–46 = 20 µm.



FIGS. 47–49. *Anatoma euglypta*. Radula and jaw plate of specimens from 57°40'S, 26°25'W, 309 m (MLP 12063). FIG. 47: Jaw plate; FIGS. 48–49: Radula; FIG. 48: General view; FIG. 49: Detail of central and lateral teeth. Scale bars FIG. 47 = 100 µm; FIG. 48 = 50 µm; FIG. 49 = 20 µm.

Anatoma euglypta resembles in general anatomy to *Anatoma yaroni* Herbert, 1986, *Thieleella reticulata* Bandel, 1998 (identified by Sasaki, 1998 as *Anatoma* sp.), and *Anatoma* sp. of Strasoldo (1991). *Anatoma euglypta* differs from *A. yaroni* in having the postero-basal expansion of tentacles (epipodial sense organ?) in the three epipodial tentacles instead only in the two latter as in *A. yaroni*, and in having three pallial tentacles instead of only one, as reported for *A. yaroni* by Herbert (1986). Five pairs of epipodial tentacles were reported for *Thieleella reticulata*, whereas only three pairs are present in *A. euglypta*. Strasoldo (1991) noted an anterior threefold tentacle, with an epipodial sense organ; a second threefold group is situated further posterior, and additionally a single tentacle on the left side is encountered.

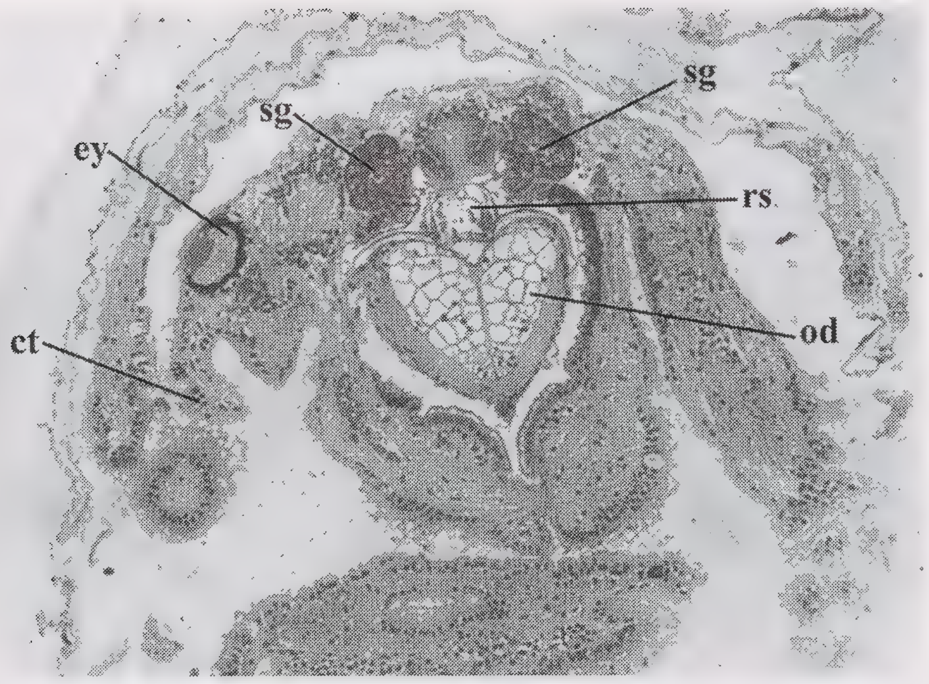


FIG. 50. Histological section of the anterior part of *Anatoma euglypta*. ct: cephalic tentacle; ey: eye; od: odontophore; rs: radular sheath; sg: salivary gland.

The morphology of the radula in *Anatoma euglypta* is very similar to that of *Anatoma baxteri* McLean, 1984 (Geiger, 2003: fig. 16F), *Anatoma yaroni* (Herbert, 1986: figs. 1–2), and *Thieleella reticulata* (Sasaki, 1998: fig. 43g, h as *Anatoma* sp.), but in the former, lateral tooth 5 is narrower and claw shaped in outline. The illustration of the radula of *A. euglypta* provided by Numanami & Okutani (1990: fig. 5; refigured in Numanami, 1996: fig. 6D) was imaged in the anterior, worn region; the more rounded tooth morphology is due to wear and is taxonomically insignificant.

Anatoma shiraseae Numanami & Okutami, 1990
Figures 51–55

Anatoma shiraseae Numanami & Okutani, 1990: 102–104, figs. 14–16; Numanami & Okutani, 1992: 172; Numanami, 1996: 20–21, figs. 9A–C.
Anatoma s.l. *shiraseae*: Geiger, 2003: 75.

Type Locality

Günnerus Bank, Antarctica [68°23.5'S, 24°07.5'E], 281–282 m (OD).

Material Examined

Holotype (NSMT-Mo 69097) and paratype (NSMT Mo. 69098).

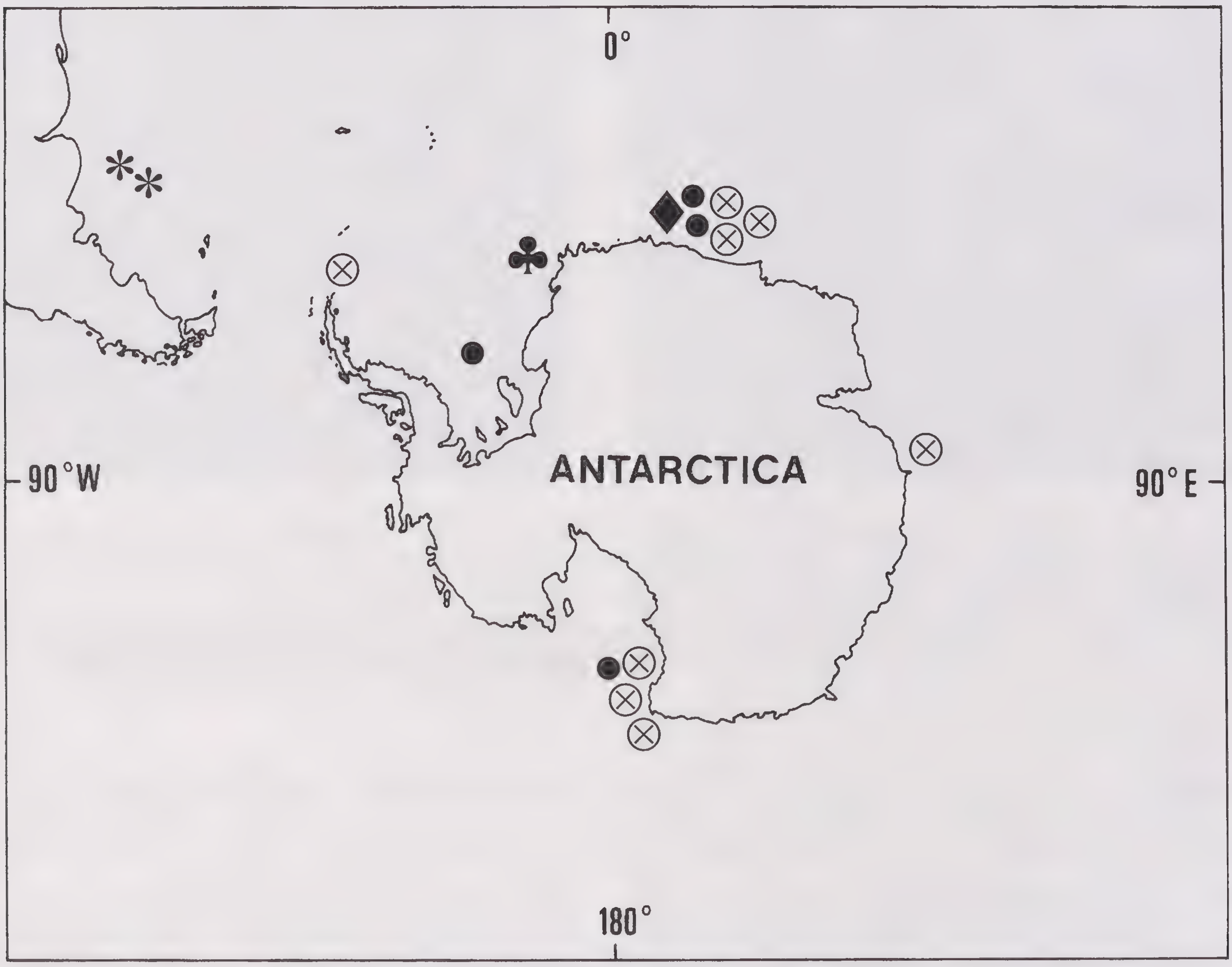


FIG. 51. Distribution of three anatomid species: *Anatoma shiraseae* (◆); *Thieleella amoena*, material examined (⊗) and bibliographic records (●); ***Thieleella weddelliana*** (♣); ***Thieleella argentinae*** (✱).

Distribution

Known only from the type locality (Fig. 51).

Etymology

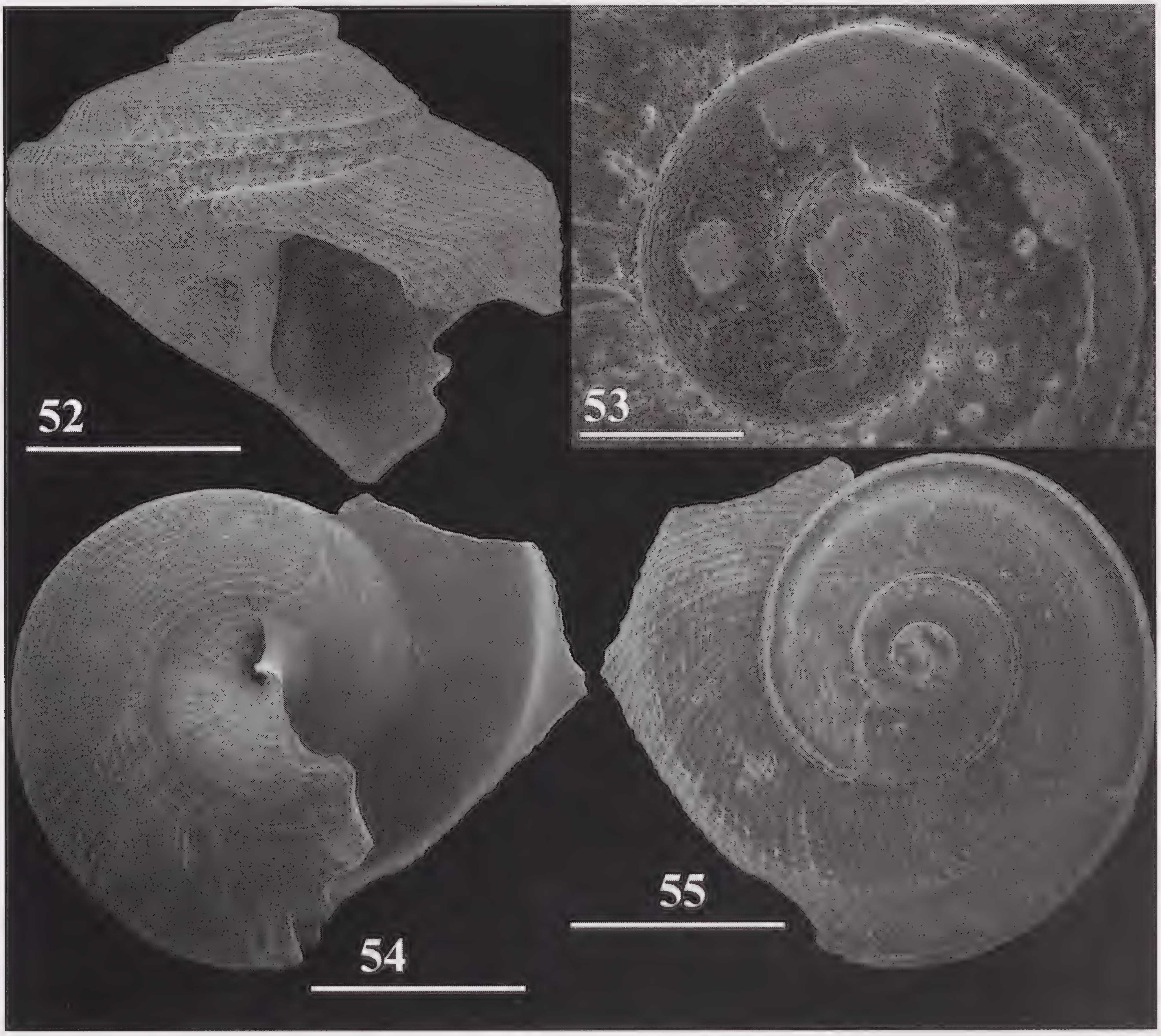
Named for the icebreaker *Shirase*.

Diagnosis

Shell large, biconical, with a prominent spiral ridge on shoulder, and denselly cancellate sculpture.

Description

Shell large (up to 4.2 mm in diameter), biconical (Fig. 52). Protoconch of 1 1/8 whorls, 244 µm in diameter in the holotype, with flocculant sculpture, rounded margin (Fig. 53), and no apertural varix. Teleoconch I of 3/4 whorls, sculptured with low axial ribs, two spiral cords in position of selenizone (Fig. 55). Teleoconch II of up to 2 1/4 whorls. Shoulder with many fine axials, approximately 95 on last whorl, intersected by approximately 21 radials of same strength, producing cancellate pattern (Figs. 52, 55). Shoulder with distinct, broad spiral ridge. Base with similar spiral ridge below selenizone (Fig. 52). Base with approximately 115 fine axials, 29 somewhat stronger spirals threads. Umbilicus wide, open, sloping continuously with base (Fig. 54). Parietal portion of aperture slightly flared; no funiculus. Aperture subquadratic. Selenizone at periphery with fragile distended keels. Slit open,



FIGS. 52–55. *Anatoma shiraseae*. Holotype (NSMT-Mo 69097). FIG. 52: Apertural view; FIG. 53: Protoconch; FIG. 54: Umbilical view; FIG. 55: Apical view. Scale bars FIGS. 52, 54–55 = 1 mm; FIG. 53 = 100 µm.

margins constricting anteriorly (Fig. 52). Operculum corneous, multispiral, slightly smaller than aperture. Animal unknown.

Remarks

The placement of the species in either *Anatoma* or *Thieleella*, distinguished by protoconch sculpture, could not be resolved from Numanami & Okutani's (1990) original description, because no SEM images of the protoconch were provided. We examined the holotype by SEM, showing flocculant protoconch sculpture (Fig. 53), which confirms its placement in *Anatoma*.

The body of the wet preserved holotype of *Anatoma shiraseae* was removed by DLG almost completely except for the very tip of the visceral mass.The uncoated shell was imaged by variable pressure SEM. The animal had distinctly recognizable eggs. The paratype shell had been coated for SEM by the original authors, but was partially broken prior to examination by SEM (K. Hasegawa, pers. comm.). The pieces show the diagnostic keel on the shoulder of the shell, but the protoconch is missing from the remains.

Thieleella Bandel, 1998

Type Species (OD): *Scissurella amoena* Thiele, 1912.

Diagnosis

Shell 2–5 mm, trochoid biconical, slit at periphery of whorl, open. Protoconch with reticulate sculpture. Reticulate sculpture is composed of small stretches of shell material varying in width by less then a factor of two, and adjacent stretches of shell material enclose a discrete area. More comprehensive diagnoses and discussions have been provided elsewhere (Geiger 2003, 2006b; Geiger & Jansen 2004a).

Remarks

Bandel (1998) erected the genus *Thieleella* for the higher than wide anatomids, with "scalar whorls", laterally placed selenizone with lamellae, and reticulate protoconch. As previously indicated by Geiger (2003), the three first characters are not exclusive for *Thieleella* and they are also present in *Anatoma*. Thus, from the original description, the only character on which *Thieleella* is based is the protoconch ornamentation: *Thieleella* has a protoconch with reticulate protoconch, whereas it is smooth or flocculant in *Anatoma*. The subtle distinction between reticulate and flocculant protoconchs was first noted by Bandel (1998), and has been confirmed by Marshall (2002), Geiger (2003, 2006b), and Geiger & Jansen (2004a).

The distributional ranges do not represent a difference between species of *Anatoma* and *Thieleella*: both genera are virtually worldwide distributed and appear from shallow to abyssal waters.

Three species of *Thieleella* for which the anatomy is known [i.e., *T. amoena*, ***T. weddelliana*** (this study), and *T. reticulata* (fide Bandel, 1998)] lack pigment in the area where eyes are usually found. This character was considered by Geiger (2003) as a potential additional character to diagnose *Thieleella*. Subsequently, Geiger (2006b) discussed and dismissed this character, because there is a member of *Thieleella*, *T. baxteri* (McLean, 1984), with distinct pigmentation in the eye region, and an *Anatoma* species, *Anatoma janetae* Geiger, 2006, seems to be functionally blind.

The examination of the radula of *Thieleella amoena* (type species) and ***T. weddelliana*** during this study did not provide any additional differences to separate this genus from *Anatoma*.

Even when more characters than those originally employed by Bandel (1998) for the description of *Thieleella* are considered, the only one that remains to separate this genus from *Anatoma* is the protoconch ornamentation. Although protoconch ornamentation is species-specific, there is clear evidence of homoplasy for particular protoconch types (Geiger, 2003). It suggests that the protoconch ornamentation found in the species assigned to *Thielleela* might not diagnose a clade, but is only one particular variation present among *Anatoma* s.l.-species. However, we prefer to conservatively retain both genera, until some additional evidence becomes available, as done by Marshall (2002), Geiger (2003), and Geiger & Jansen (2004a).

Thieleella amoena (Thiele, 1912)

Figures 51, 56–70

Scissurella amoena Thiele, 1912a: 187, pl. 11, fig. 11; Thiele, 1912b: 9, pl. 1, fig. 17.

Schizotrochus amoena [*sic*]: Carcelles, 1953: 161.

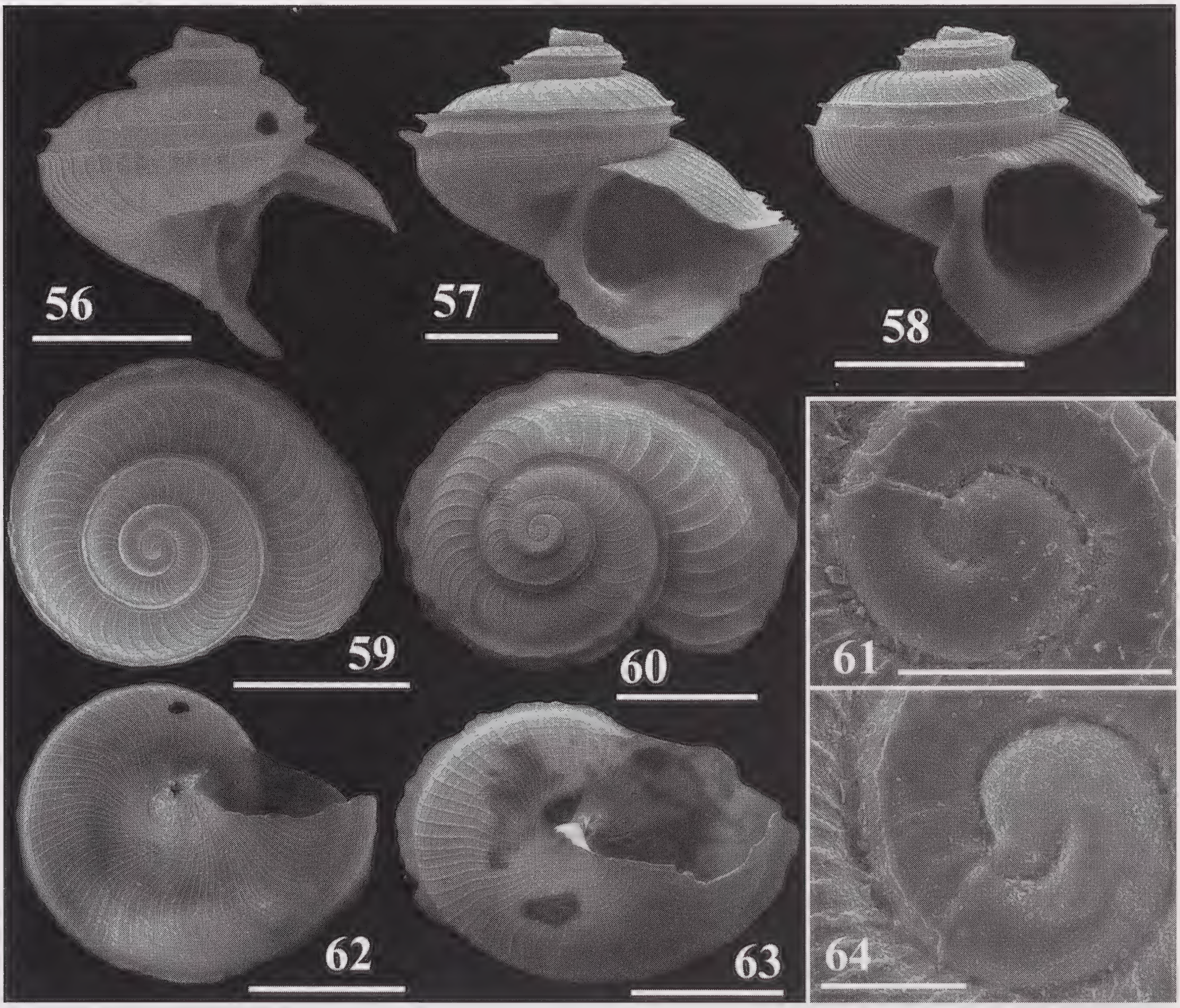
Schizotrochus amoenus: Powell, 1960: 126; Egorova, 1982: 11, fig. 71; Ageitos de Castellanos & Landoni, 1988: 10.
Anatoma amoena: Lozouet, 1986: 112; Dell, 1990: 75, fig. 130; Numanami & Okutani, 1990: 100–102, figs. 9–13; Numanami & Okutani, 1992: 172; Branch et al., 1991: 47, 58, 62; Numanami, 1996: 18–19, figs. 8A–E.
Thieleella amoena: Bandel, 1998: 35, pl. 11, figs. 7–8; pl. 12, fig. 1; Geiger, 2003: 74.

Type Locality

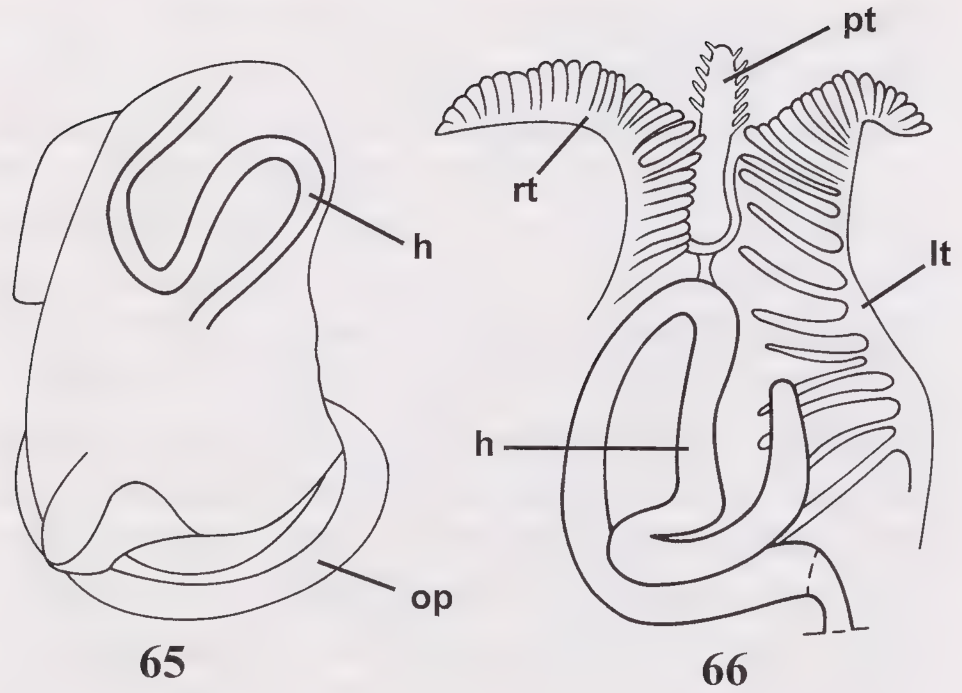
Gauss Station [Davis Sea], Antarctica [66°S, 89°E] (OD).

Material Examined

Lectotype (ZMB-Moll 63047-a) (here designated) and 2 paralectotypes (ZMB-Moll 63047-b). Weddell Sea: 62°46.73’S, 53°02.57’W, 1579–1584 m (16 sps & 4 shs, ZSM Mol 20062197). Ross Sea: 72°05’S, 172°15’E, 392 m (8 shs, USNM 1084418); 72°05’S, 172°15’E, 392 m (4 shs, USNM 1084418); 72°60’S, 172°28’E, 530–548 m (2 shs, NMNZ M.242840). East Antarctica: Lazarev Sea: 69°43’S, 10°46’E, 432 m (11 shs, SMNH 83287); 69°59’S, 08°00’E, 161–185 m (6 shs, SMNH 83288; 7 shs, SMNH 83289; 1 sh, 83290); 69°57’S, 06°20’E, 280–298 m (5 sps, SMNH 83291).



FIGS. 56–64. *Thieleella amoena*. FIG. 56: Lectotype; FIGS. 57, 60, 63: Specimen from 62°46.73’S, 53°02.57’W, Weddell Sea, 1579–1584 m (ZSM Mol 20062197); FIGS. 58, 61: Specimen from 72°05’S, 172°15’E, Ross Sea, 392 m (USNM 613010); FIGS. 59, 62, 64: Specimen from 72°60’S, 172°28’E, Ross Sea, 530–548 m (NMNZ M.242840); FIGS. 56–58: Apertural view; FIGS. 59–60: Apical view; FIGS. 61, 64: Protoconch; FIGS. 62–63: Umbilical view. Scale bars FIGS. 56–60, 62–63 = 1 mm; FIG. 61 = 200 µm, FIG. 64 = 100 µm.



FIGS. 65–66. *Thieleella amoena*. Anatomy. FIG. 65: Detail of hindgut; FIG. 66: Detail of the pallial cavity roof (inner view). h: hindgut; lt: left tentacle; op: operculum; pt: pallial tentacle; rt: right tentacle. Scale bars = 1 mm.

Other Literature Records

Weddell Sea (Bandel, 1998); Ross Sea: 72°08'S, 172°10'E, 433 m (Dell, 1990); East Antarctica: Breid Bay: 70°10'S, 24°E, 271–310 m (Numanami & Okutani, 1990); 70°09.1'S, 24°01.9'E, 295–310 m (Numanami, 1996).

Distribution

Circumantarctic. 161–1584 m (living specimens: 280–1584 m) (Fig. 51).

Etymology

Amoena: (L.) adjective for pleasant, delightful.

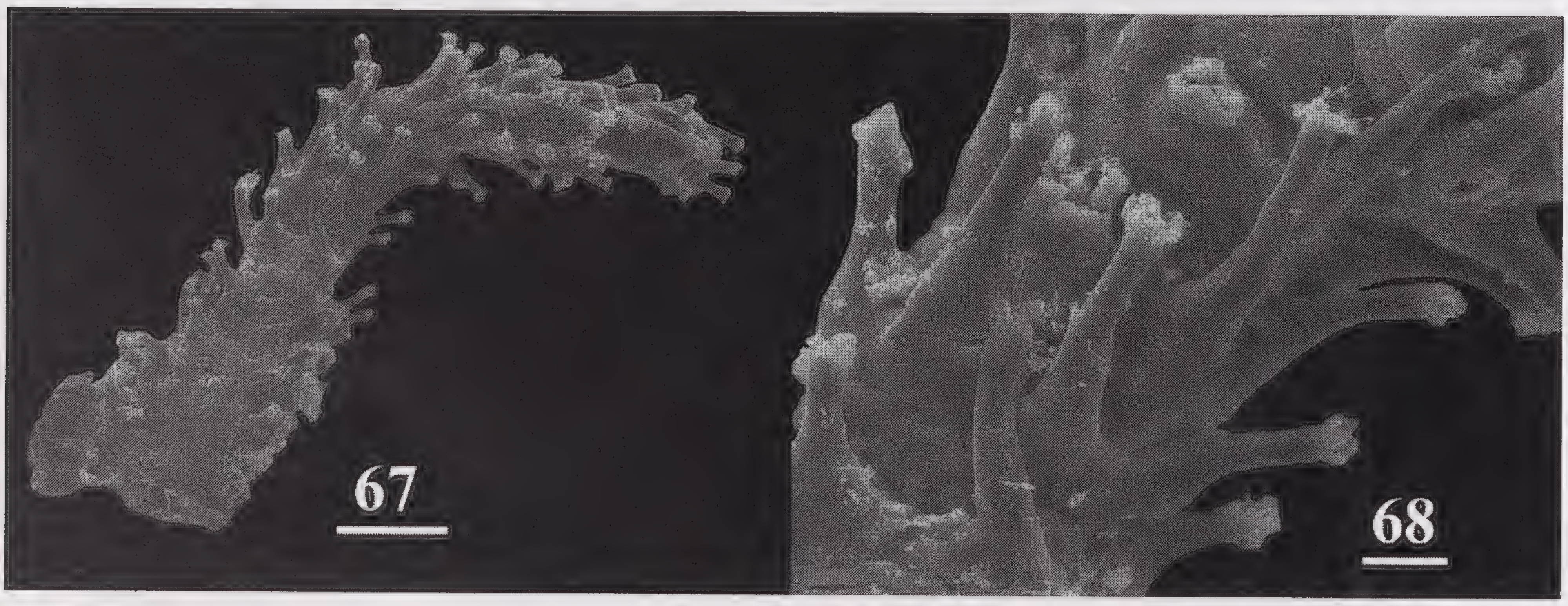
Diagnosis

Shell globose, with reticulate protoconch, strong axial sculpture on teleoconch II, and flared lower portion of aperture.

Description

Shell medium size (up to 3.5 mm in diameter), broad trochiform, broader than high (Figs. 56–58). Protoconch of 3/4 to 1 whorl, 176–196 µm in diameter, with reticulate sculpture; apertural varix not connected to embryonic cap; apertural margin convex (Figs. 61–64). Teleoconch I of one whorl, with 22–27 axial ribs; spiral cord in position of selenizone forming strong angulation (Figs. 59–60). Teleoconch II with up to 2 1/2 whorls; shoulder with 37–39 axial ribs on first whorl, 38–47 cords on second whorl (Figs. 59–60). Base with spirals somewhat stronger than on shoulder, but weaker than axial ribs (Figs. 62–63). Selenizone at periphery of shell, with elevated keels; slit open, with parallel margins. Umbilicus open (Figs. 62–63).

Head-Foot: Whitish, uniform. Cephalic tentacles long, circular, and papillate. Eyes, eyestalks absent. Neck wide, with two smooth tentacles on each side. Three pairs of long, sparsely micropapillate epipodial tentacles, present; first epipodial tentacle bifurcated at base with one additional short posterior pteriform appendix (epipodial sense organ?); second and third tentacles simple. Papillae tubular, about 27 to 33 µm in length, spirally



FIGS. 67–68. *Thieleella amoena*. Details of epipodial tentacles and papillae. Scale bars FIG. 67 = 50 µm; FIG. 68 = 10 µm.

arranged, with distal cillia (Figs. 67–68). Pallial cavity with two large bipectinate ctenidia, left one of about 10% larger (Fig. 66). A relatively long, papillate, conical tentacle extending anteriorly, starting in posterior part of mantle sinus (Fig. 66). Hindgut forming inclined “S” (Fig. 65).

Radula (Figs. 69–70): Rachidian tooth trapezoidal at base, with nearly straight cups, showing main central denticle, 5 narrower denticles on each side. Five pairs of lateral teeth; laterals 13 similar in outline, overlapping at their bases; cusps elongate, tongue-like, with one prominent main denticle, 4–6 denticles on outer margin, 3–4 narrower denticles on inner margin; fourth lateral tooth narrower than laterals 1–3, with more delicate serration at cusp; lateral tooth fifth wide at base, with prominent main denticle and strongly serrated margins in cusp. Marginal teeth long, narrow, main denticle straight sharp, numerous serrations on both sides.

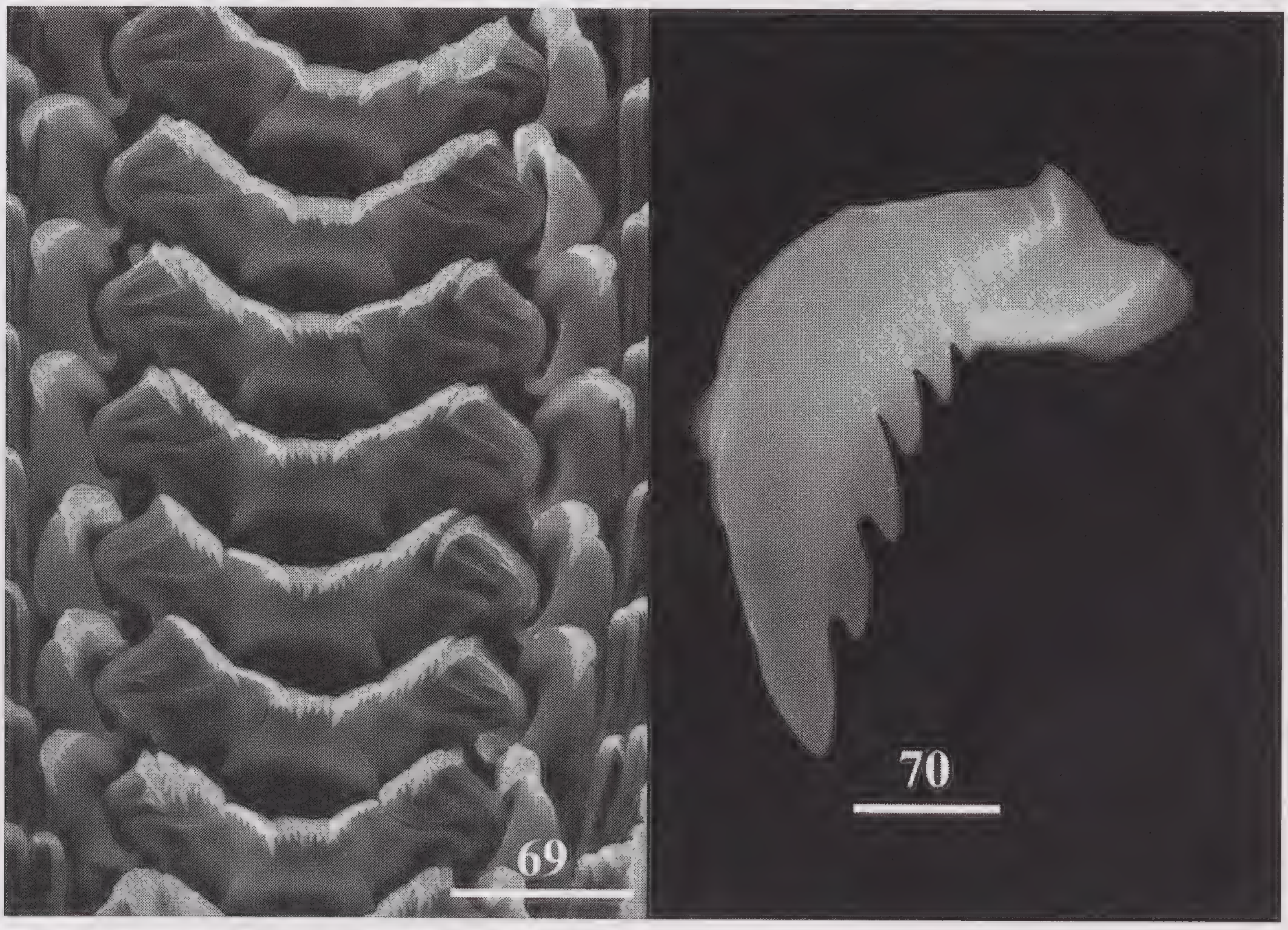
Remarks

Because *Thieleella amoena* is the type species of the genus, we select here one of the three syntypes as lectotype (Fig. 56) with the express taxonomic purpose to fix the species concept.

The most similar species to *Thieleella amoena* is *Anatoma conica*. They share the overall globose shell, sculptured with strong axial ribs. However, *T. amoena* has fewer and comparatively narrower axial ribs, a wider selenizone, and an inclined protoconch in apertural view, with flocculant sculpture. In addition, *T. amoena* lacks the flared lower portion of the apertural lip. Furthermore, the two species seem to be allopatric: *A. conica* occurrs in the Magellan Region, whereas *T. amoena* occurs in Antarctic waters.

Thieleella gunteri (Cotton & Godfrey, 1933) from eastern Australia has noticeably stronger sculpture on the shoulder compared to the base, more numerous and fainter spirals, and lacks the flared aperture.

Anatoma shiraseae from Antarctica has a distinct keel on the shoulder, a distinct constriction below the selenizone, and a protoconch with flocculant sculpture. The other Antarctic anatomid species, *Anatoma euglypta*, has a biconical shell that is not tur-reted, a hardly sunken suture, an extremely fine and dense axial and even finer spiral sculpture, and a protoconch with flocculant sculpture.



FIGS. 69–70. *Thieleella amoena*. Radula of a specimen from 62°46.73’S, 53°02.57’W, 1579–1584 m (ZSM Mol 20062197). FIG. 69: General view; FIG. 70: Detail of lateral tooth 5. Scale bars FIG. 69 = 20 µm; FIG. 70 = 2 µm.

Thieleella weddelliana n. sp.

Figures 51, 71–84

Type Locality

70°31.08’S, 14°34.83’W, Weddell Sea, 4384–4420 m.

Type Material

Holotype (ZSM Mol 20062198) and 2 paratypes (ZSM Mol 20062199).

Distribution

Known only from the type locality (Fig. 51).

Etymology

The species is named after the type locality.

Diagnosis

Thieleella weddelliana is characterized by having a large, laterally expanded shell, with rounded whorls, wide and deep umbilicus, and strong axial ribs.

Description

Shell large (up to 5.6 mm in diameter), trochiform, laterally expanded (Fig. 71). Protoconch of one whorl, about 220 µm in diameter, with reticulate ornamentation; margin of protoconch convex, without varix (Fig. 74). Teleoconch I of 3/4 to 5/6 rounded whorls, sculptured with 10–12 axial ribs, one spiral cord in position of selenizone forming strong angulation; teleconch II of up to 2 3/4 whorls (Fig. 73). Whorls of spire rounded; last whorl nearly straight; shoulder and base convex. Shell with cancellate sculpture; axial ribs strong: 22–26 on shoulder of first whorl, 27–28 on second whorl, 31–41 on base; spiral threads weak, beginning at start of selenizone, gradually increasing in number with growth: 5–8 on first whorl, 25–30 at apertural margin (Figs. 71–73). Selenizone displaced towards suture, sculptured with low axial ribs, surrounded by narrow, strong keels (Fig. 71). Umbilicus open, wide, deep (Fig. 72); funiculus present. Aperture rounded.

Anatomy: Animal pale cream, uniform. Cephalic tentacles long, lanceolate, flat, sparsely papillate; papillae tubular, of about 110 µm long, spirally arranged, slightly expanded distally with a hadfull of cilia (Figs. 80, 83–84). Eyes and eyestalks absent. Head separated from foot by wide neck, with one pair of smooth cylindrical tentacles on each side. Three pairs of conical papillate epipopial tentacles; first epipodial tentacle divided basally forming two tentacles. Papillae similar to that of cephalic tentacles, but smaller (42–50 µm long) (Figs. 81–82). Columellar muscle thick, elongate in outline. Pallial cavity with enlarged mantle border, deeply sinuated in medial line, one rounded papillate tentacle extending anteriorly in medial line (Fig. 78). Two large bipectinate ctenidia; left ctenidium about 10% larger than right. Hindgut very large, sinuous, drawing posteriorly directed loop at left side, before ending in anus (Figs. 78–79).

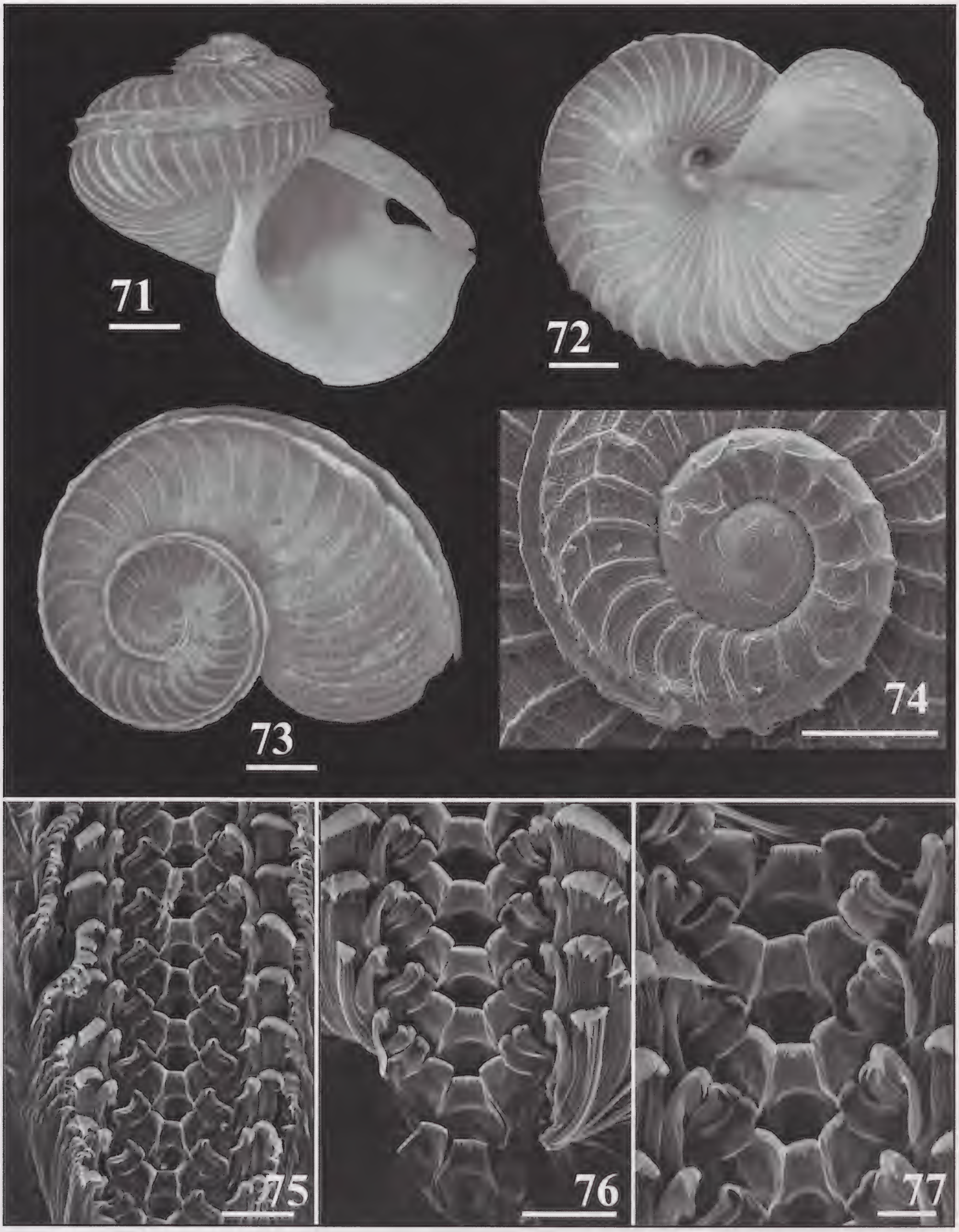
Radula (Figs. 75–77): Rachidian tooth trapezoidal at base, cusp nearly straight, with one main central denticle and 6–10 narrower, sharper denticles on each side. Five pairs of lateral teeth; laterals 1–3 with rectangular bases, innermost tooth widest; cusp with prominent innermost denticle, 7–11 narrower denticles on outer margin; inner margin smooth. Fourth lateral tooth narrower than lateral 3; lateral tooth 5 claw-shaped, wider at base, with wide, sharply serrated cusp. Marginal teeth numerous, long, narrow, becoming more delicate outwards, with prominent, delicatelly serrated cusp.

Remarks

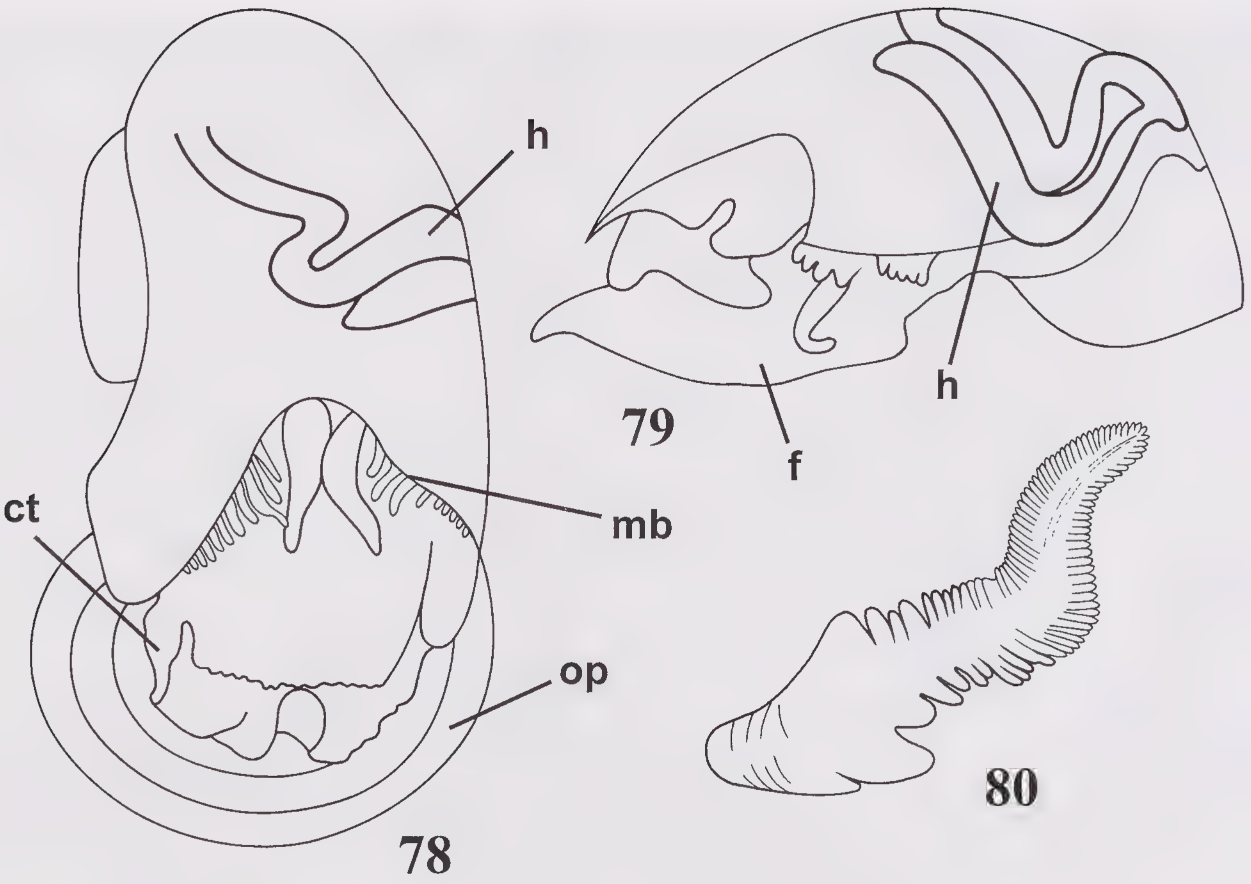
Thieleella weddelliana resembles *Thieleella amoena* and *Anatoma conica*, from which it differs in having a larger shell, with wider umbilicus and selenizone displaced towards the suture. Furthermore, the last whorl is more uniformely rounded in *A. conica* and *T. amoena* than in ***T. weddelliana***. In addition, ***T. weddelliana*** has fewer axial ribs in the first whorl of teleoconch II than *T. amoena* (22–26 vs. 37–39).

Thieleella weddelliana differs from *Anatoma euglypta* and *Anatoma shiraseae*, the other two Antarctic species of anatomids, in having a globular instead of a biconical last whorl, and stronger axial sculpture.

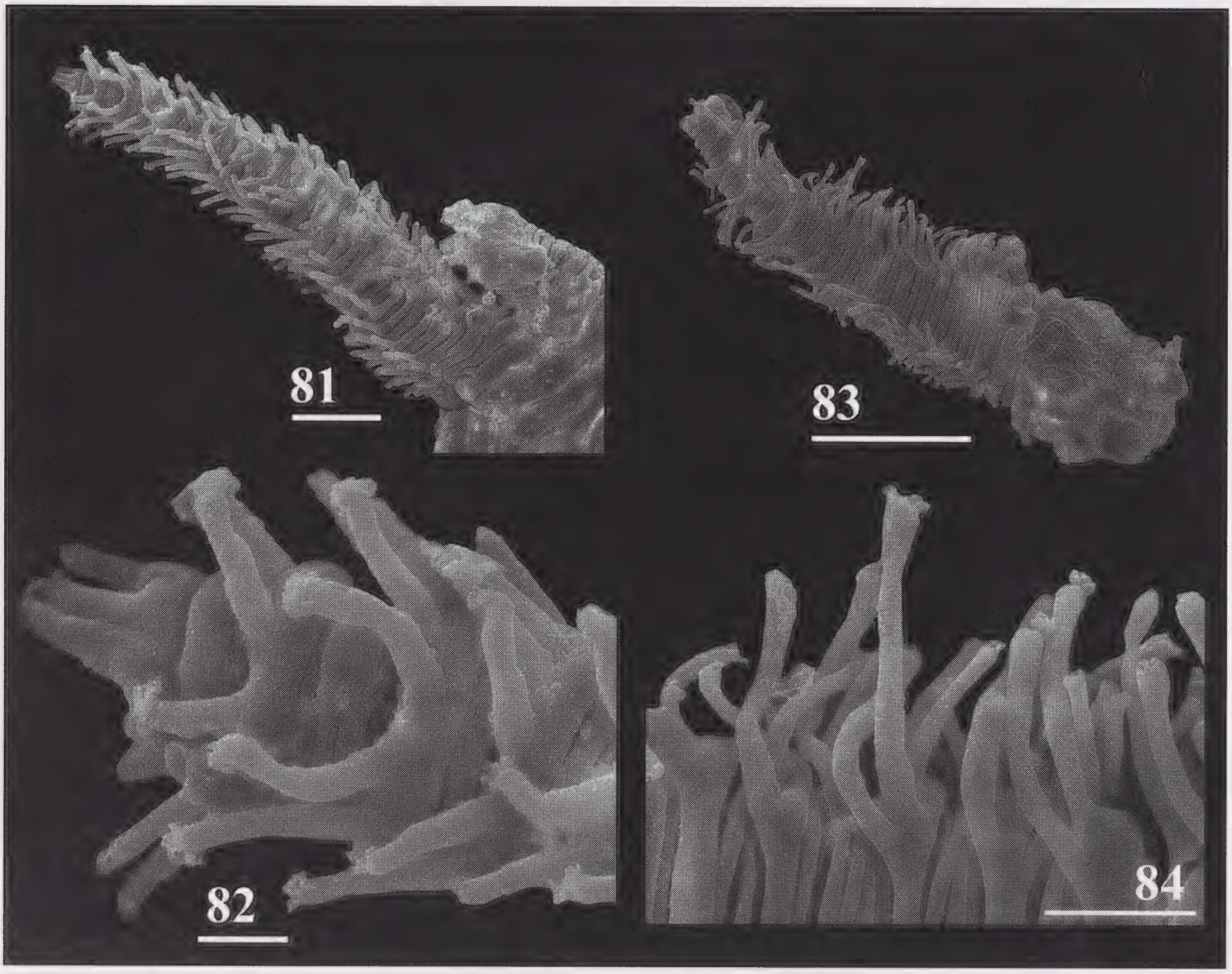
Thieleella baxteri (McLean, 1984) from the northeastern Pacific is similar in overall shape,



FIGS. 71–77. ***Thieleella weddelliana***. FIGS. 71–73: Holotype (ZSM Mol 20062198); FIGS. 74–77: Paratypes; FIG. 71: Apertural view; FIG. 72: Umbilical view; FIG. 73: Apical view; FIG. 74: Protoconch; FIGS. 75–77: Radula. Scale bars FIGS. 71–73 = 1 mm; FIG. 74 = 200 µm; FIGS. 75–76 = 50 µm; FIG. 77 = 20 µm.



FIGS. 78–80. ***Thieleella weddelliana***. Anatomy. FIGS. 78–79: Detail of hindgut (h); FIG. 78: Frontal view; FIG. 79: Lateral view; FIG. 80: Detail of cephalic tentacle. ct: cephalic tentacle; f: foot; mb: mantle border; op: operculum.



FIGS. 81–84. ***Thieleella weddelliana***. FIGS. 81–82: Epipodial tentacle; FIG. 81: General view; FIG. 82: Detail of papillae; FIGS. 83–84: Cephalic tentacle; FIG. 83: General view; FIG. 84: Detail of papillae. Scale bars FIG. 81 = 100 μm; FIG. 82 = 20 μm; FIG. 83 = 250 μm; FIG. 84 = 50 μm.

but has more numerous (30 vs. 13) weaker axial ribs on teleoconch I, and lacks a spiral cord in the position of the selenizone on teleoconch I. The differences in the density and strength of the sculpture are similar on teleoconch II. As the spiral threads on shoulder and base are of equal strength in both species, *T. baxteri* appears more reticulate as spirals and axials are more equal in strength, whereas in ***T. weddelliana***, the axial ribs are much more prominent than the spirals.

The reticulate protoconch sculpture of this species is composed of smaller and more irregular cells than in other *Thieleella* species. The observed sculpture is best described as reticulate, which is diagnostic for *Thieleella*. The sculpure of ***T. weddelliana*** cannot be described as the flocculent type frequently encountered in *Anatoma* spp. As discussed elsewhere (Geiger, 2003), the single character is provisionally considered sufficient to diagnose the genus, though confirmation through other lines of evidence is desirable.

Thieleella argentinae n. sp.
Figures 51, 85–89

Type Locality

43°33'S, 48°58.1'W, Argentine Basin, 5208–5223 m, stn 247, Atlantis II-60 Expedition.

Type Material

Holotype (MCZ 356975, operculum and radula on SEM stub, shell); 15 paratypes from type locality (MCZ 345737; broken shells with fluid preserved animals); 3 paratypes (MCZ 345380; small shells with fluid preserved animals), 36°55.7'S, 53°01.4'W, Argentine Basin, 2707 m, stn 245, Atlantis II-60 Expedition.

Distribution

36°55.7'–43°33'S, 53°01.4'–48°58.1'W, Argentine Basin, 2707–5223 m (Fig. 51).

Etymology

Named for its provenance.

Diagnosis

A depressed and widely umbilicated *Thieleella*, with a large protoconch of about 480 µm in diameter, and cancellate shell sculpture.

Description

Shell large (up to ~ 5 mm in diameter), trochiform, broader than high (Fig. 85). Protoconch of one whorl, globose, about 480 µm in diameter, with reticulate sculpture; apertural margin convex, with prominent varix (Fig. 86). Teleoconch I of 5/8 whorls, sculptured with 11 axial ribs; spiral cord in position of selenizone forming angulation (Fig. 88). Teleoconch II of 1 1/4 whorls at 1.3 mm (broken in the aperture); shoulder with 57 axial ribs in the first whorl, and narrower spiral threads than increase in number and solidness with growth. Base with prominent spirals surrounding umbilicus. Umbilicus open, wide (Fig. 87). Selenizone at periphery, with elevated keels; condition of slit unknown. Operculum corneous, multispiral, slightly smaller than aperture (Fig. 89).

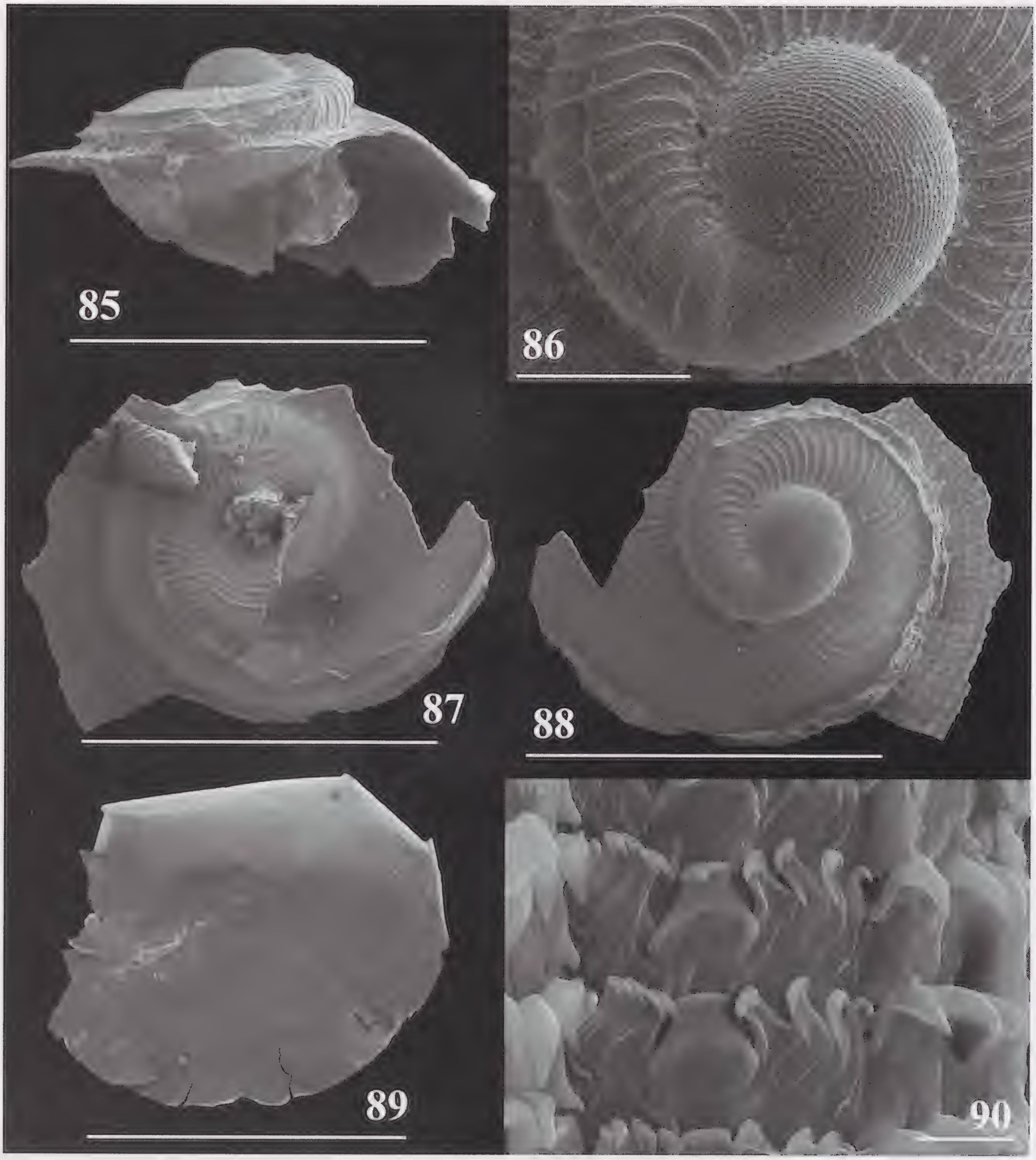
Anatomy: Cephalic tentacles long, papillate; eye and eyestalks absent; two neck tentacles, present; first epipodial tentacle with three branches, second with two.

Radula (Fig. 90): Rachidian tooth pyriform at base; cutting edge straight, with one main central denticle and 4–5 smaller, sharper denticles on each side. Five pairs of lateral teeth; laterals 1–3 relatively wide at bases, with triangular cusps, bearing one main apical denticle and 3–4 narrower denticles on outer margin; fourth lateral tooth narrower and smaller; lateral tooth 5 strong, with wide base, sharply serrated inner margin of cusp. Marginal teeth numerous; inner marginals similar to lateral tooth 5; outer marginals more delicate with finely denticulated cusps.

Remarks

Thieleella argentinae is most similar to *Anatoma josephinae* Odhner, 1960, described from the Sargasso Sea (28°N), 6000 m depth. However, ***T. argentinae*** has a somewhat larger protoconch (480 µm vs. 357 µm) with reticulate sculpture, which is inflated in the region of the embryonic cap, while in *A. josephinae* the protoconch shows the typical spiral coiling. In addition ***T. argentinae*** has a teleoconch I of 1/4 whorls, sculptured with 11 axial ribs, while *A. josephinae* has 3/5 whorls and 18 axials ribs.

The available specimens of ***Thieleella argentinae*** have only the early portion of the teleoconch I along with the protoconch preserved. Although the specimens are very incomplete, they clearly belong to a new species.



FIGS. 85–90. ***Thieleella argentinae***. Holotype (MCZ 356975). FIG. 85: Apertural view; FIG. 86: Protoconch; FIG. 87: Umbilical view; FIG. 88: Apical view; FIG. 89: Operculum; FIG. 90: Radula. Scale bars FIGS. 85, 87–89 = 1 mm; FIG. 86 = 200 µm; FIG. 90 = 20 µm.

Scissurellidae Gray, 1847

Diagnosis

Shell 0.6–3 mm, trochoid, more or less depressed; protoconch sculpture variable; slit above periphery, open (*Scissurella*, *Incisura*), closed (*Ariella*, *Sinezona*, *Satondella*, *Incisura*), or absent (*Coronadoa*); radula with lateral tooth 5 enlarged by broadening.

Remarks

Anatomidae have the slit or foramen at the periphery and lateral tooth 5 is enlarged by elongation. More comprehensive diagnoses and discussions have been provided elsewhere (Geiger, 2003, 2006a, b; Geiger & Jansen, 2004a, 2004b).

Scissurella d'Orbigny, 1824

Type Species (SD: Gray, 1847): *Scissurella laevigata* d'Orbigny, 1824.

Diagnosis

Shell 0.6–2 mm, trochiform depressed, many protoconch ornamentations except reticulate and microhexagonal; slit above periphery, open. More comprehensive diagnoses and discussions have been provided elsewhere (Geiger, 2003; 2006a, b; Geiger & Jansen, 2004b).

Scissurella clathrata Strebel, 1908
Figures 91–120

Scissurella clathrata Strebel, 1908: 77–78, pl. 6, fig. 84a–d; Thiele, 1912b: 9, pl. 1, figs. 18–19; Powell, 1960: 126; Ageitos de Castellanos & Landoni, 1988: 7, pl. 1, fig. 3; Linse, 1997: 27; Linse & Brandt, 1998: 883; Linse, 1999: 400.

Scissurella eucharista Melvill & Standen, 1912: 344, pl., fig. 1–1a; Powell, 1960: 126; Ageitos de Castellanos & Landoni, 1988: 6, pl. 1 fig. 2.

Schizotrochus clathrata [*sic*]: Carcelles & Williamson, 1951: 251.

Schizotrochus eucharista [*sic*]: Carcelles & Williamson, 1951: 251.

Anatoma clathrata: Lozouet, 1986: 112; Linse, 2002: 64–67 [in part], fig. 4.2–2, pl. 1b, figs. 9.1.1.5–9.1.1.8.

Scissurella/Anatoma? clathrata: Geiger, 2003: 76.

Scissurella obliqua Watson: Geiger, 2003: fig. 16e [*non* Watson, 1886].

Type Localities

54°43'S, 64°08'W, Feuerland [= Tierra del Fuego, Argentina], 36 m (*Scissurella clathrata*) (OD). 54°25'S, 57°32'W, Burdwood Bank, 56 ft [102 m] (*Scissurella eucharista*) (OD).

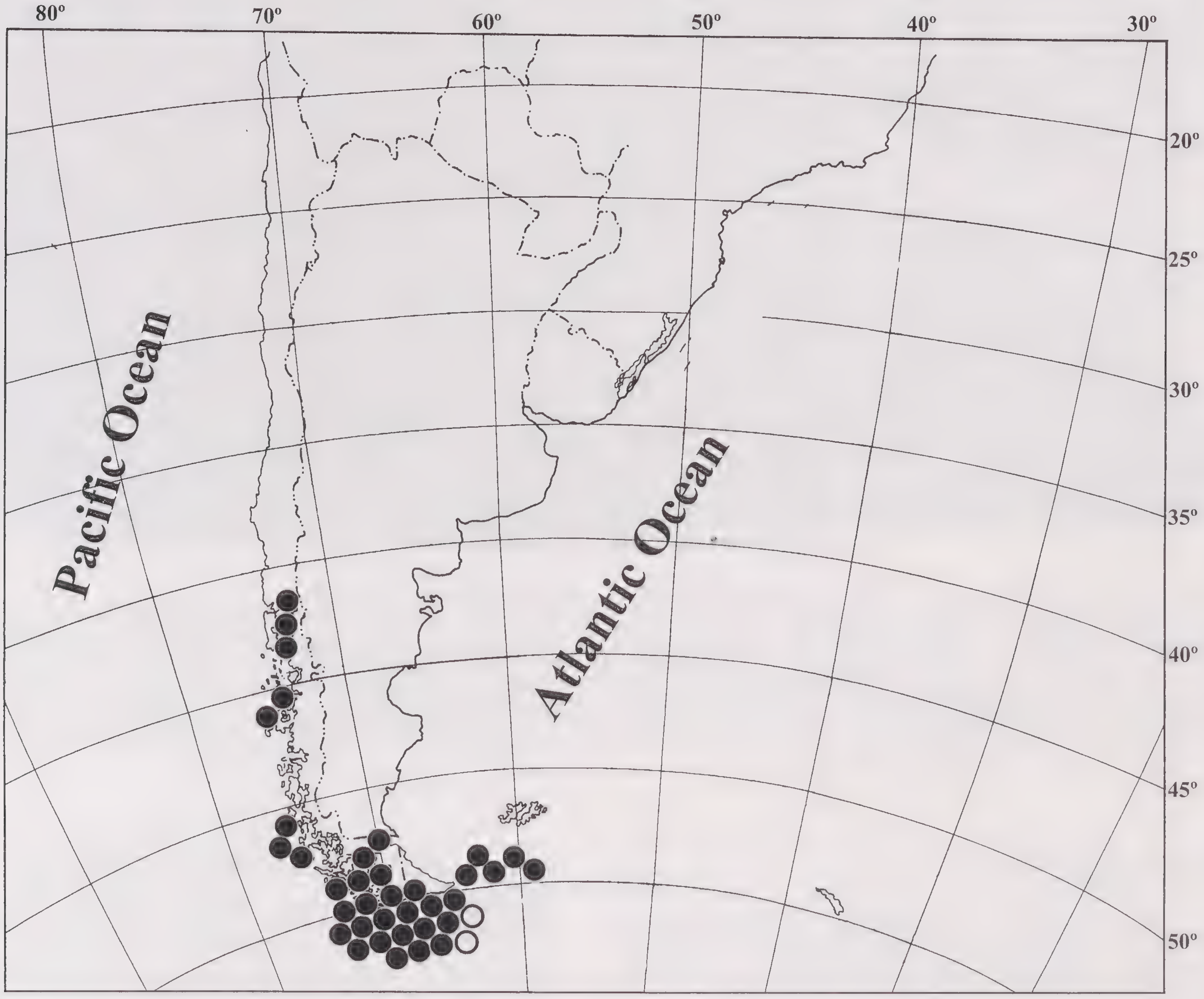
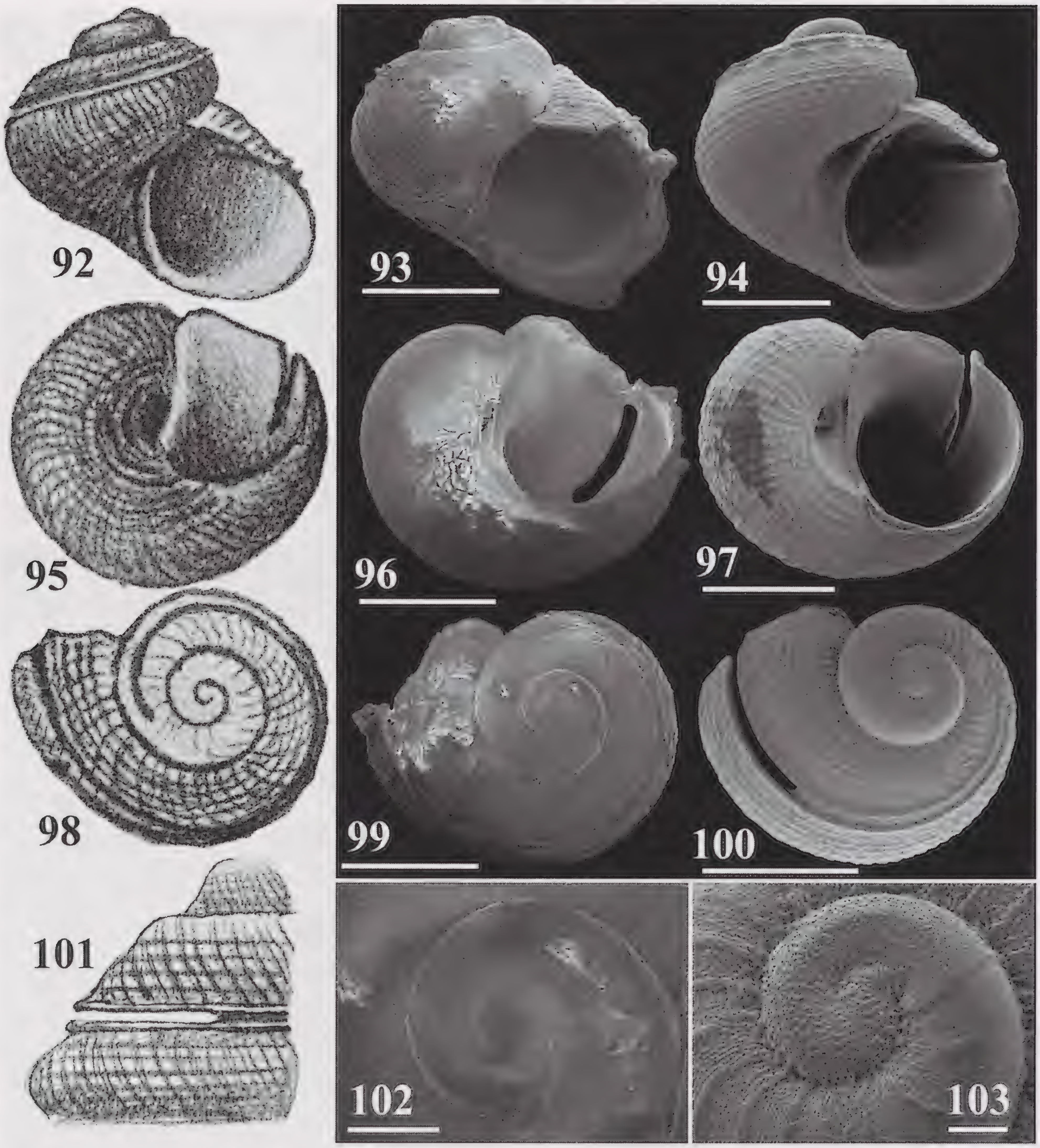


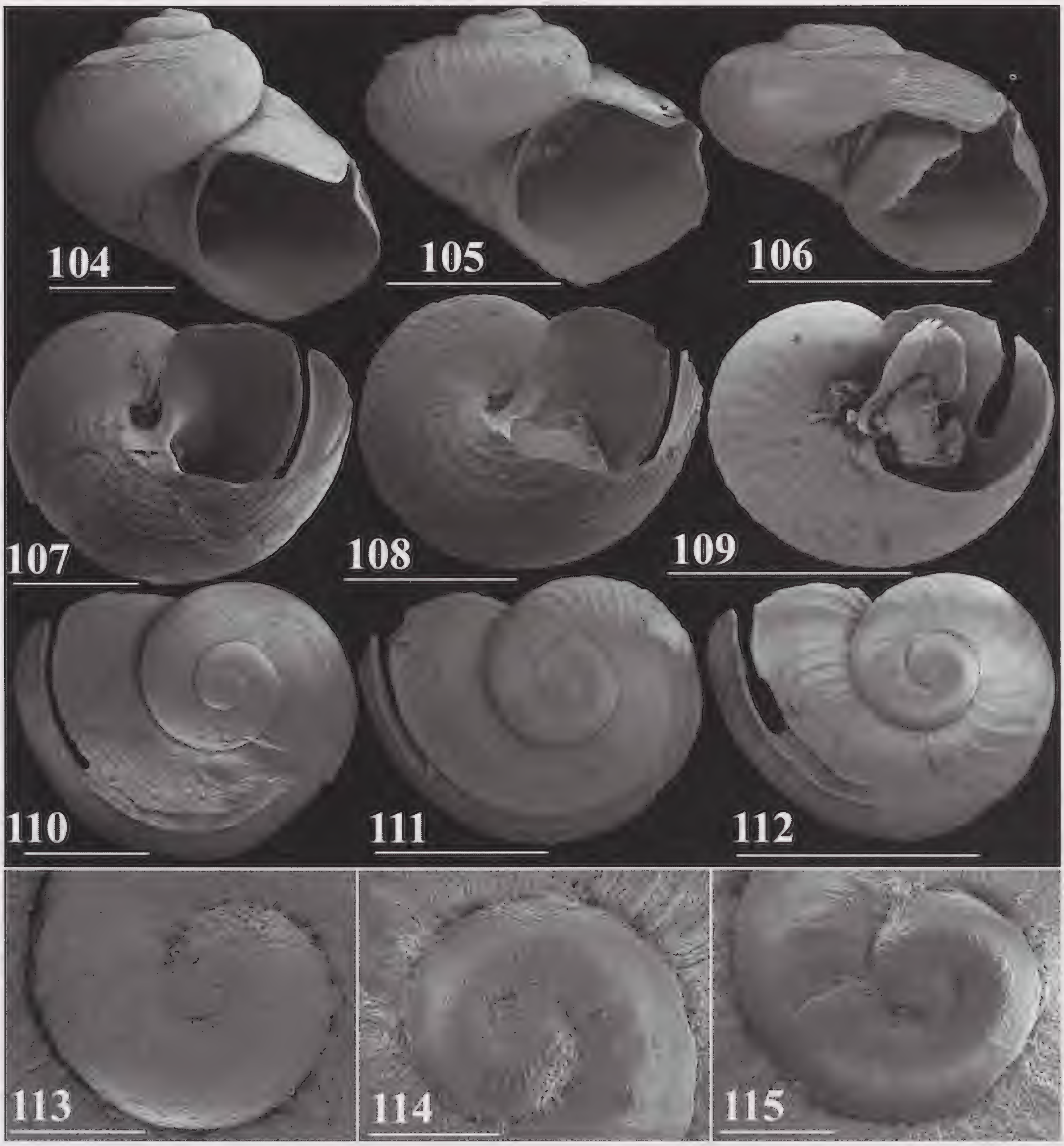
FIG. 91. Distribution of *Scissurella clathrata*. ●: material examined; o: bibliographic records.



FIGS. 92–103. *Scissurella clathrata*. FIGS. 92, 95, 98, 101: Original figures of *Scissurella clathrata* by Strebel (1908); FIGS. 93, 96, 99, 102: Holotype of *Scissurella eucharista* (NMSZ 1921.143.595); FIGS. 94, 97, 100, 103: Specimen from 55°03'S, 66°37'W, 30–35 m (MLP 12079); FIGS. 92–94: Apertural view; FIGS. 95–97: Umbilical view; FIGS. 98–100: Apical view; FIG. 101: Detail of selenizone and shell sculpture; FIGS. 102–103: Protoconch. Scale bars FIGS. 93–94, 96–97, 99–100 = 500 µm; FIG. 102 = 100 µm; FIG. 103 = 50 µm.

Material Examined

Holotype of *Scissurella eucharista* (NMSZ 1921.143.595). Southern Argentina: 54°48'S, 65°16'W, W side of Bahía Buen Suceso, 0 m (8 sps & 3 shs, LACM 71-270); Isla de los Estados: 54°43'S, 64°14'W, Isla Conett, 17 m (1 sh, LACM 73-66); 54°34'S, 64°10'W, Isla Observatorio, 73 m (4 shs, LACM 71-316); 54°34'S, 64°30'W, Cabo San Antonio, 73–76 m (16 shs, LACM 71-352). Beagle Channel: 55°07'18"S, 66°52'47"W, Punta Rico, 25 m (26 sps & 10 shs, ZMH 19589); 55°38'31"S, 67°12'52"W, Isla Wollaston, 40 m (39 sps &



FIGS. 104–115. *Scissurella clathrata*. Ontogenetical variability. FIGS. 92, 104, 107, 110, 113: Specimen from 43°14'S, 73°36'W, E side Isla Laitec, 3–6 m (LACM 75-46); FIGS. 105, 108, 111, 114: Specimen from 43°54'S, 73°43'W, Westhoff Island, 22 m (LACM 73-75); FIGS. 106, 109, 112, 115: Specimen from 45°27'S, 74°24'W, Canal Darwin, 9 m (LACM 73-74); FIGS. 104–106: Apertural view; FIGS. 107–109: Umbilical view; FIGS. 110–112: Apical view; FIGS. 113–115: Protoconch. Scale bars FIGS. 104–112 = 500 μm; FIGS. 113–115 = 100 μm.

127 shs, ZMH 19591). 55°00'31''S, 66°53'08''W, Isla Gardiner, 103 m (1 sp, ZMH 19594); 54°52'S, 68°13'W, 12–20 m (1 sh, MLP 12068); 54°50'S, 68°15'W, 18–21 m (1 sp, MLP 12069); 54°50'S, 68°20'W, 5 m (4 shs, MLP 12070); 54°50'S, 68°19'W, 5 m (2 shs, MLP 12071); 54°52'S, 68°14'W, 7–13 m (3 sps, MLP 12072); 54°51'S, 68°19'W, 5–10 m (1 sh, MLP 12073); 54°52'S, 68°07'W, 21–23 m (1 sp, MLP 12074); 54°51'S, 68°02'W, 27 m (2 sps, MLP 12075); 54°55'S, 67°21'W, 15–20 m (6 sps & 6 shs, MLP 12076); 54°57'S, 67°01'W, 30–40 m (15 sps, MLP 12077); 55°02'S, 66°42'W, 1520 m (2 sps & 1 sh, MLP

12078); 55°03'S, 66°37'W, 30–35 m (22 sps. and 28 shs MLP 12079); 55°06'S, 66°29'W, 65–80 m (6 sps & 3 shs, MLP 12080); 55°06'50"S, 66°55'32"W, Isla Picton, 110 m (1 sp, ZMH 19590); SE of Isla Picton: 55°06'53"S, 66°39'57"W, 63 m (218 sps & 204 shs, ZMH 19592); 55°07'36"S, 66°44'36"W, 33 m (3 sps, ZMH 19593). Magellan Strait: 52°57'30"S, 70°41'00"W, Bahía Laredo, 90 m (1 sp, ZMH 19587); 54°22'S, 71°21'W Punta Valparaíso, Canal Cockburn, 18 m (13 shs, LACM 73-69); 52°24'S, 73°39'W, Punta Dashwood, Canal Smyth, 13 m (11 shs, LACM 73-71); Península Brunswick: 53°37'S, 70°56'W, Puerto Hambre, S of Punta Askew, 0 m (3 shs, LACM 75-49); 53°38'S, 70°54'W, Punta Santa Ana, Fuerte Bulnes, 0 m (2 shs, LACM 75-45). Southern Chile: 43°54'S, 73°43'W, Westhoff Island, 22 m (40 shs, LACM 73-75); 46°35'S, 75°30'W, Bahía San Andres, N of Golfo de Peñas, 1 m (13 shs, LACM 73-73); 43°14'S, 73°36'W, E side of Isla Laitec, Chiloé Island, 3–6 m (20 shs, LACM 75-46); 42°38'S, 72°57'W, Islota Nihuel, Golfo Corcovado, Chiloé Province, 3–15 m (4 shs, LACM 75-43); 45°27'S, 74°24'W, Canal Darwin, 9 m (29 shs, LACM 73-74); 50°11'S, 74°48'W, Bahía Tom, 15 m (80 sps, LACM 73-72); 52°56'S, 75°00'W, 92–101 m (3 shs, NMNZ M.242830); 52°40'S, 74°58'W, 64 m (2 shs, NMNZ M.242829).

Other Literature Records

Beagle Channel: 55°07.3'S, 66°53.0'W, Punta Rico, 25 m (Linse, 2002); 55°07.2'S, 66°40.2'W, SE of Isla Picton, 67 m (Linse, 2002).

Distribution

Magellan Region. 0–110 m (living over entire bathymetric range) (Fig. 91).

Etymologies

Clathrata: (L.) referring to the cancellate sculpture. *Eucharista*: *Eucharistos* (G.) for elegant, agreeable.

Diagnosis

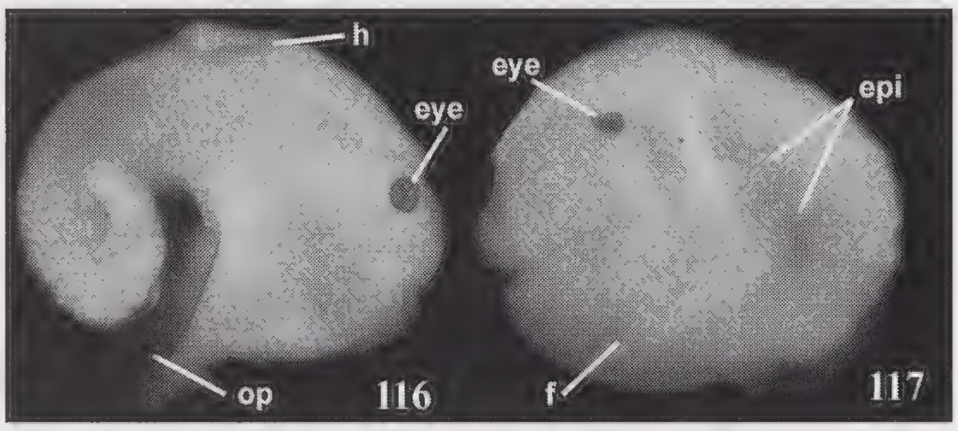
Shell inflated with densely cancellate sculpture.

Description

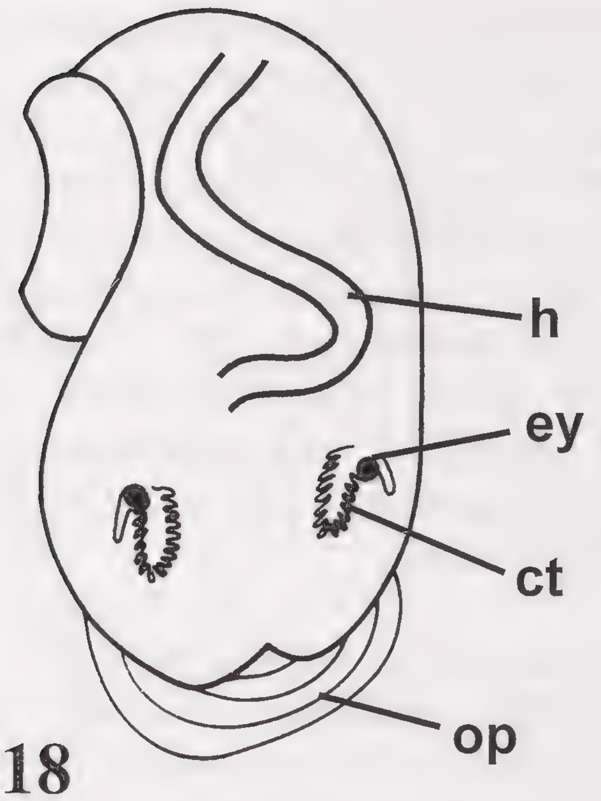
Shell of medium size (up to 1.4 mm in diameter), trochiform, inflated (Figs. 92–94, 104–106). Protoconch of 1 to 1 1/8 whorls, 190–250 µm in diameter, with flocculant sculpture; apertural margin convex, without varix (Figs. 102–103, 113–115). Teleoconch I of 7/8–1 whorl, sculptured with 24 delicate spiral cords, 24–39 axial ribs; interstices with irregular growth marks (Figs. 98–100, 110–112). Teleoconch II of up to 1 1/8 whorls, with convex shoulder; juveniles laterally expanded, mature specimens with inflated base (Figs. 104–106); sculpture cancellate, consisting in 37–45 low, irregularly spaced axial ribs, 16–19 spirals cords, forming small nodules at intersections (Figs. 92–101, 104–112). Selenizone above periphery, surrounded by low keels having low growth marks; slit open, with parallel margins (Figs. 92–94, 104–106). Umbilicus moderately narrow, bordered by carina of smooth walls (Figs. 95–97, 107–109). Aperture round, roof overhanging.

Anatomy (Figs. 116–119): Color pale cream, uniform. Pallial cavity with two large bipectinate ctenidia, left one of about 10% larger. Cephalic tentacles short, flat, foliated, papillate. Eyestalks short, fused at base of cephalic tentacles; eyes black, lens half of eye in diameter. Three pairs of conical, relatively long, papillate epipopial tentacles; first epipodial tentacle divided basally forming two tentacles, with posterior branch bifurcated distally; second and third tentacles simple. Foot long, narrow; foot sole completely ciliated. Columellar muscle thick, triangular in outline. Single small tentacle located at postero-dorsal part of mantle cavity. Salivary glands elongate, larger than odontophore. Intestine very long, with hindgut forming wide inverted "S".

Radula (Figs. 120–122): Rachidian tooth with broadly triangular base and nearly straight



FIGS. 116–117. *Scissurella clathrata*. Anatomy. FIG. 116: Lateral view; FIG. 117: Ventral view. epi: epipodial tentacle; eye: eye; f: foot; h: hindgut; op: operculum.



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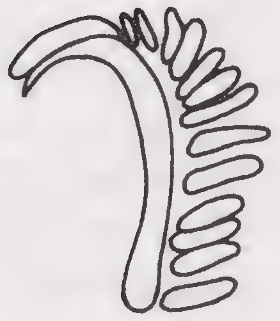

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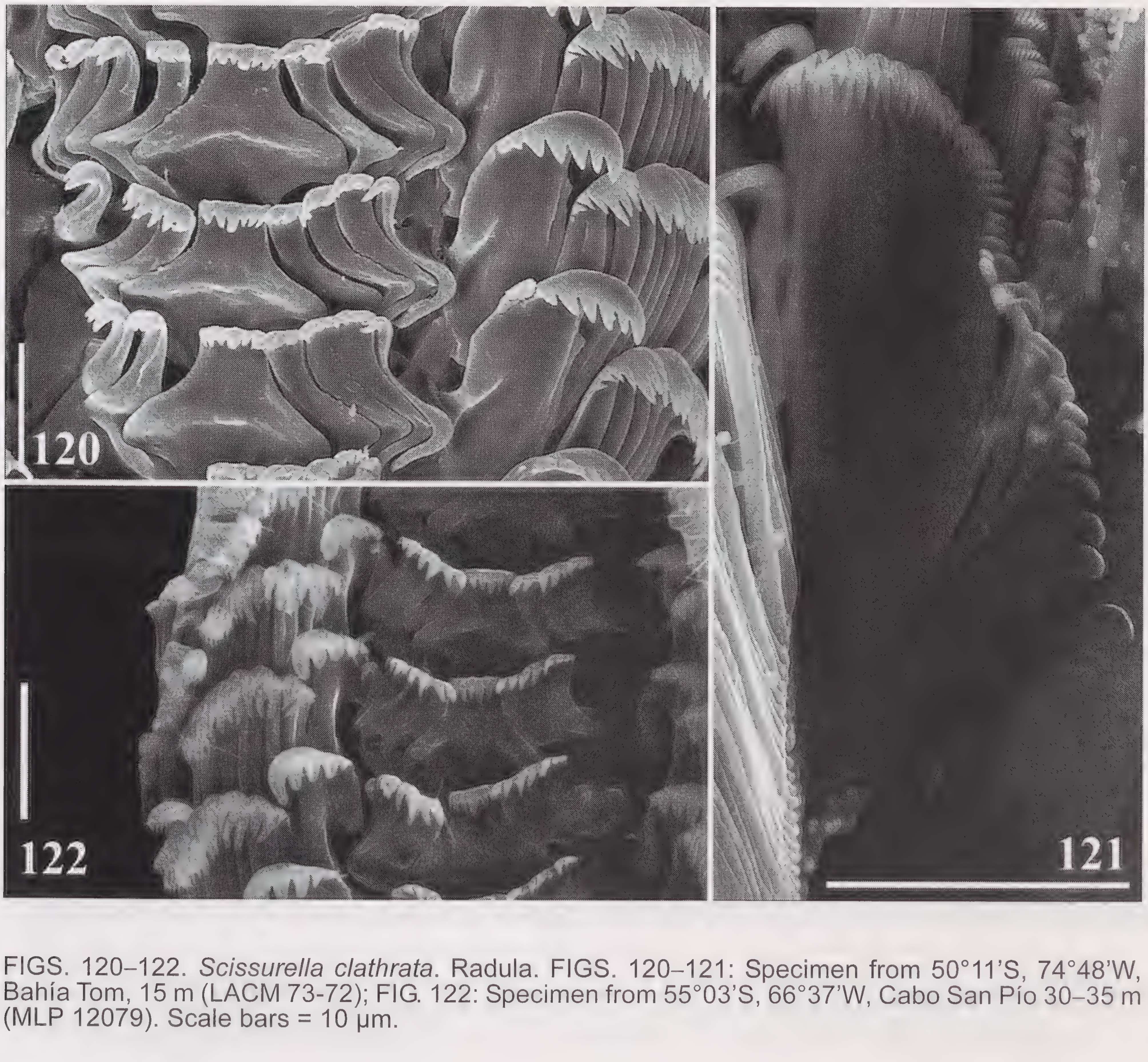
FIGS. 118–119. *Scissurella clathrata*. Anatomy. FIG. 118: Hindgut. ct: cephalic tentacle; ey: eye; h: hindgut; op: operculum. FIG. 119: Ctenidium.

cusp, with a main central denticle and 3 narrower denticles on each side. Lateral teeth 1–3 similar; cusp with three denticles on outer margin. Lateral tooth 4 reduced, with one central denticle on cusp, and one smaller denticle on each side. Lateral tooth 5 wider, larger than other laterals, with 7 strong, rounded at tip, denticles on inner margin; outer margin smooth. Radular interlock of central field moderate. Inner marginal teeth with main terminal denticle, three denticles on inner margin, four denticles on outer margin; outer marginals spoon shaped.

Remarks

The holotype of *Scissurella clathrata* could not be located at SMNH, although SMNH records place it there (K. Sindemark, 3/2006, pers. comm.), nor at ZSM (Zelaya, 9/2006,



FIGS. 120–122. *Scissurella clathrata*. Radula. FIGS. 120–121: Specimen from 50°11'S, 74°48'W, Bahía Tom, 15 m (LACM 73-72); FIG. 122: Specimen from 55°03'S, 66°37'W, Cabo San Pío 30–35 m (MLP 12079). Scale bars = 10 µm.

pers. obs.), where other material of Strebel is housed. However, the species is clearly recognizable from the original description and figures (Figs. 92, 95, 98, 101).

Linse (2002) described the protoconch of this species as smooth and placed *clathrata* in *Anatoma* on the basis of this character. However, Geiger (2003) showed that protoconch sculpture is very variable and not suitable for generic diagnoses in scissurellids. In addition, Linse's (2002: fig. 9.1.1–7) figure of the protoconch shows evidence of erosion. The examination of numerous additional specimens during this study reveals that the protoconch of this species is not actually smooth but has flocculant sculpture (Figs. 102–103, 113–115).

The radula of the material here studied as well as that illustrated by Linse (2002: pl. 1, fig. 9.1.1–8) shows that the cusp of lateral tooth 5 is serrated only on the inner margin. This radular characteristic clearly indicates that the species belongs to Scissurellidae and not Anatomidae; the open slit places the species in the genus *Scissurella*.

Linse (2002) reported *Scissurella clathrata* from 53°28.8'S, 70°21.9'W, Estrecho, P. Ancho, 92 m. Material from this locality identified as *S. clathrata* by Linse (ZMH 19588) is here re-identied as *Anatoma conica*.

A specimen of *Scissurella clathrata* was found at raised beaches from Río Ovando, Southern Tierra del Fuego. This area was dated in 4,100 years before present, and is located 3 m over the present sea level (Zelaya & Gordillo, unpubl. data).

Scissurella petermannensis Lamy, 1910
Figures 123–146

Scissurella petermannensis Lamy, 1910: 323–324, pl. 1, figs. 14–16; Lamy, 1911: 16, pl. 1, figs. 14–16; Thiele, 1912b: 9–10, pl. 4, figs. 11–13; Arnaud, 1972b: 430; Powell, 1960: 126; Lozouet, 1986: 108; Geiger, 2003: 77.

Scissurella timora Melvill & Standen, 1912: 345, pl. 48, fig. 2, 2a; Powell, 1951: 61; Powell, 1960: 126; Castellanos, 1980: 136; Ageitos de Castellanos & Landoni, 1988: 8–9, pl. 1, fig. 1; Linse, 1999: 400; Geiger, 2003: 78.

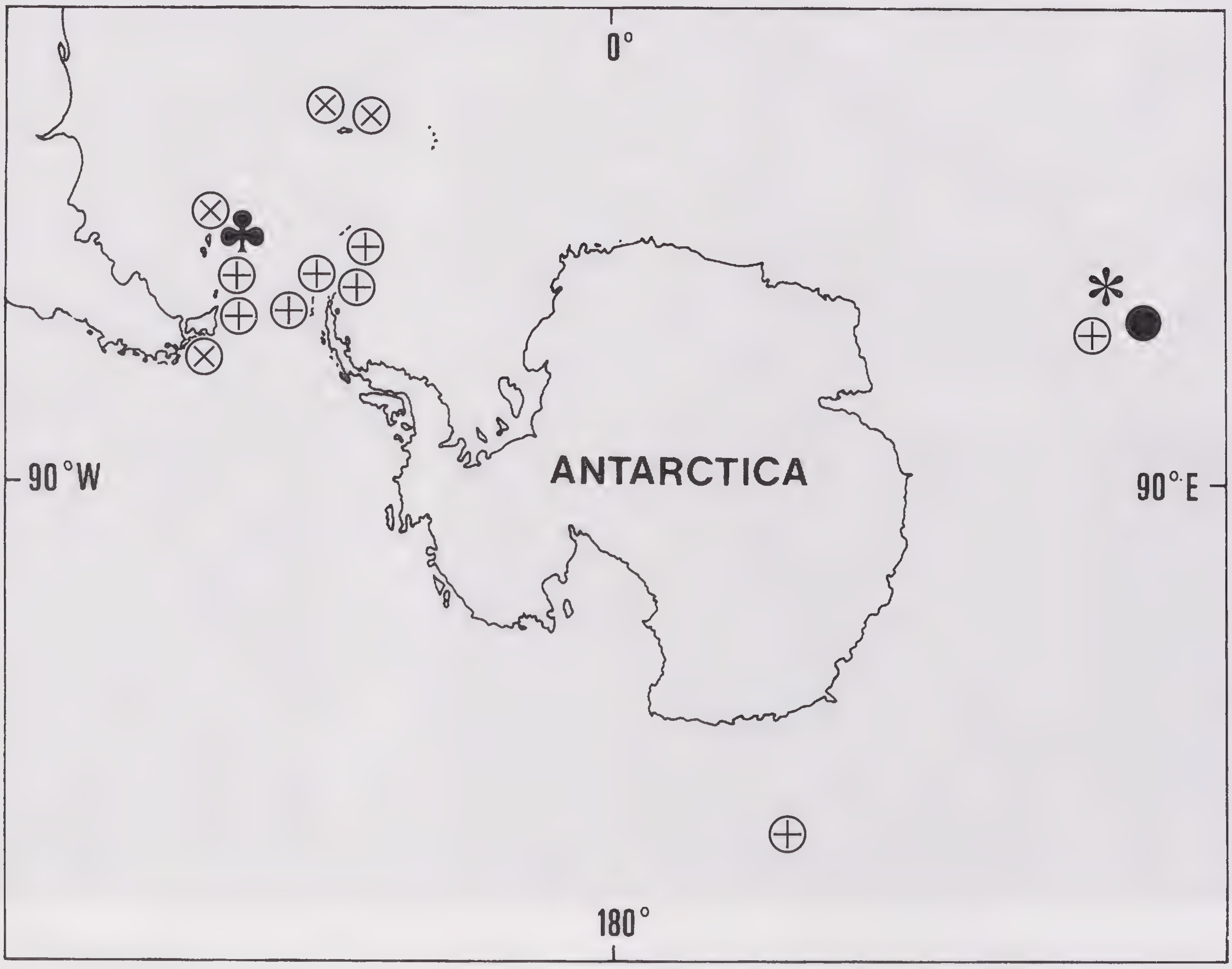


FIG. 123. Distribution of four species of *Scissurella: S. petermannensis* material examined (⊕), bibliographic records (⊗); *S. obliqua* (✱); *S. supraplicata* (●); and **S. malvinarum** (♣).

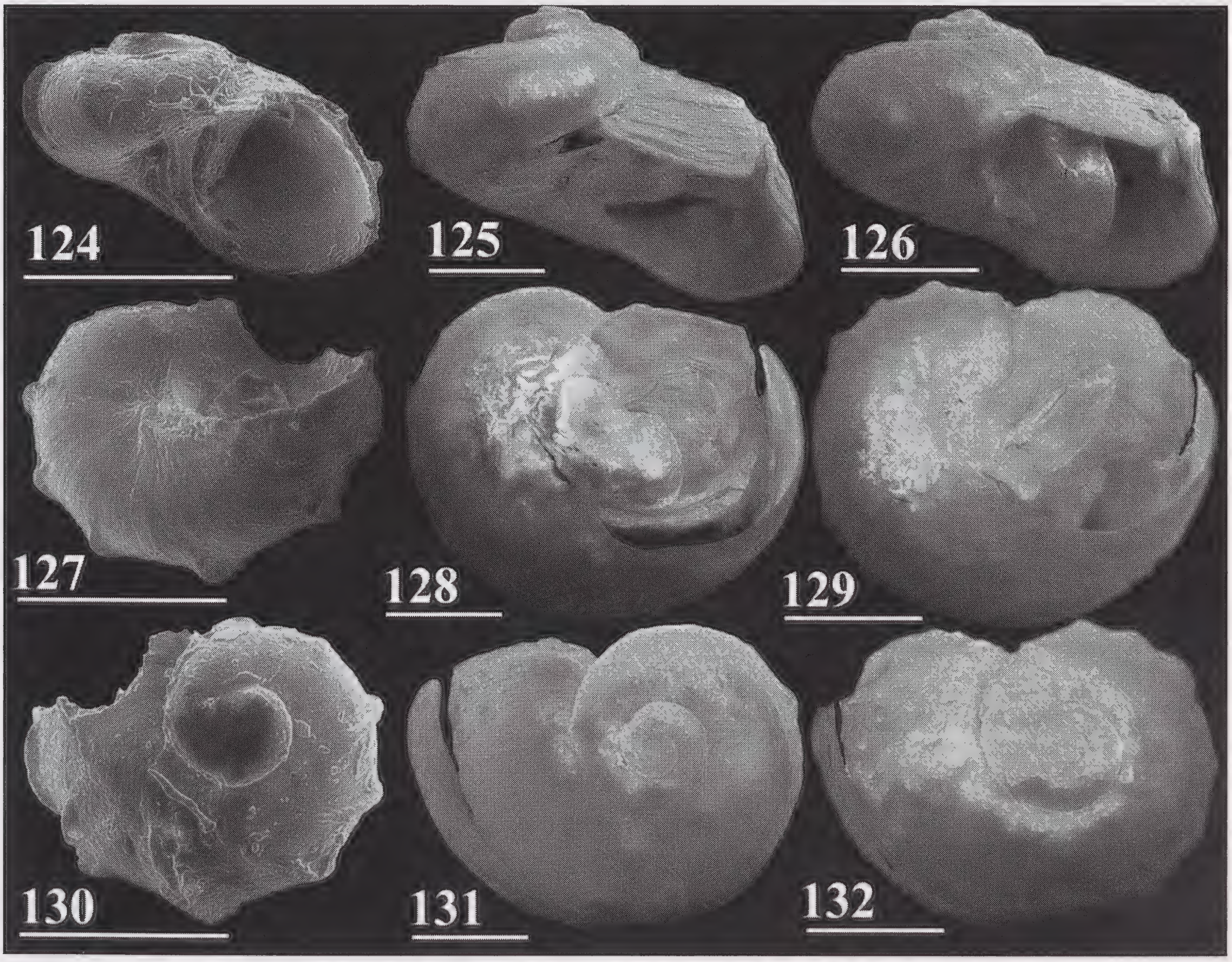
Scissurella medioplicata Thiele, 1925: 42, pl. 13, figs. 3–4; Powell, 1960: 126.
Schizotrochus petermanensis: Carcelles, 1953: 162, pl. 1, fig. 7.
Schizotrochus timora: Carcelles, 1953: 161, pl.1, fig. 6.
Scissurella cf. petermannensis: Davolos & Moolenbeek, 2005: 135, figs. 9a–d.
Scissurella cf. medioplicata: Davolos & Moolenbeek, 2005: 134, figs. 7a–d.

Type Localities

Petermann Island [65°11'S, 64°10'W, Antarctic Peninsula] (*Scissurella petermannensis*) (OD); Scotia Bay, South Orkneys 9–10 fathoms [16–18 m] (stn 325, Scottish National Antarctic Expedition) (*Scissurella timora*) (OD); Gazelle-Bay, Kerguelen Islands (stn 160, R/V Valdivia) [49°30'S, 69°30'E] (*Scissurella medioplicata*) (OD).

Material Examined

Holotype of *Scissurella petermannensis* (MNHN); lectotype (here designated) (NMSZ 2006109) and two paralectotypes (NMSZ 1921.143.598; NMW 1955.158.091) of *Scissurella timora*; lectotype (here designated) (ZMB-Moll 109.047) and one paralectotype (ZMB-Moll 109.046) of *Scissurella medioplicata*. Beagle Channel: 2 shs, 54°51'S, 68°19'W, 5–10 m (MLP 12081). Southern Argentina: 54°47'S, 64°04'W, Puerto Vancouver, Isla de los Estados, 31 m (3 sps, LACM 71-259). South Shetland Islands: 62°13.8'S, 58°40.2'W, King George Island (1 sh, ZMH 37995). Antarctic Peninsula: Palmer Station [67°42'S, 64°00'W], 1 m (1 sh, NMNZ M.221630). Macquarie Island: 54°38'S, 158°55'E, Green Gorge (1 sh, AMS C.376623).



FIGS. 124–132. *Scissurella petermannensis*. FIGS. 124, 127, 130: Holotype of *Scissurella petermannensis* (MNHN); FIGS. 125, 128, 131: Lectotype of *Scissurella timora* (NMSZ 2006109); FIGS. 126, 129, 131: Paralectotype of *Scissurella timora* (NMSZ 1921.143.598). Scale bars = 500 µm.

Other Literature Records

Malvinas Island and Strait of Magellan (Castellanos, 1980). South Georgia: 54°22'S, 36°20'W, Cumberland East Bay; 54°20'S, 36°14'W, Penguin Bay, 0.25–1 m (Davolos & Moolenbeek, 2005).

Distribution

Probably circumantarctic, reaching the southern tip of South America. 0.25–18 m (Fig. 123).

Etymologies

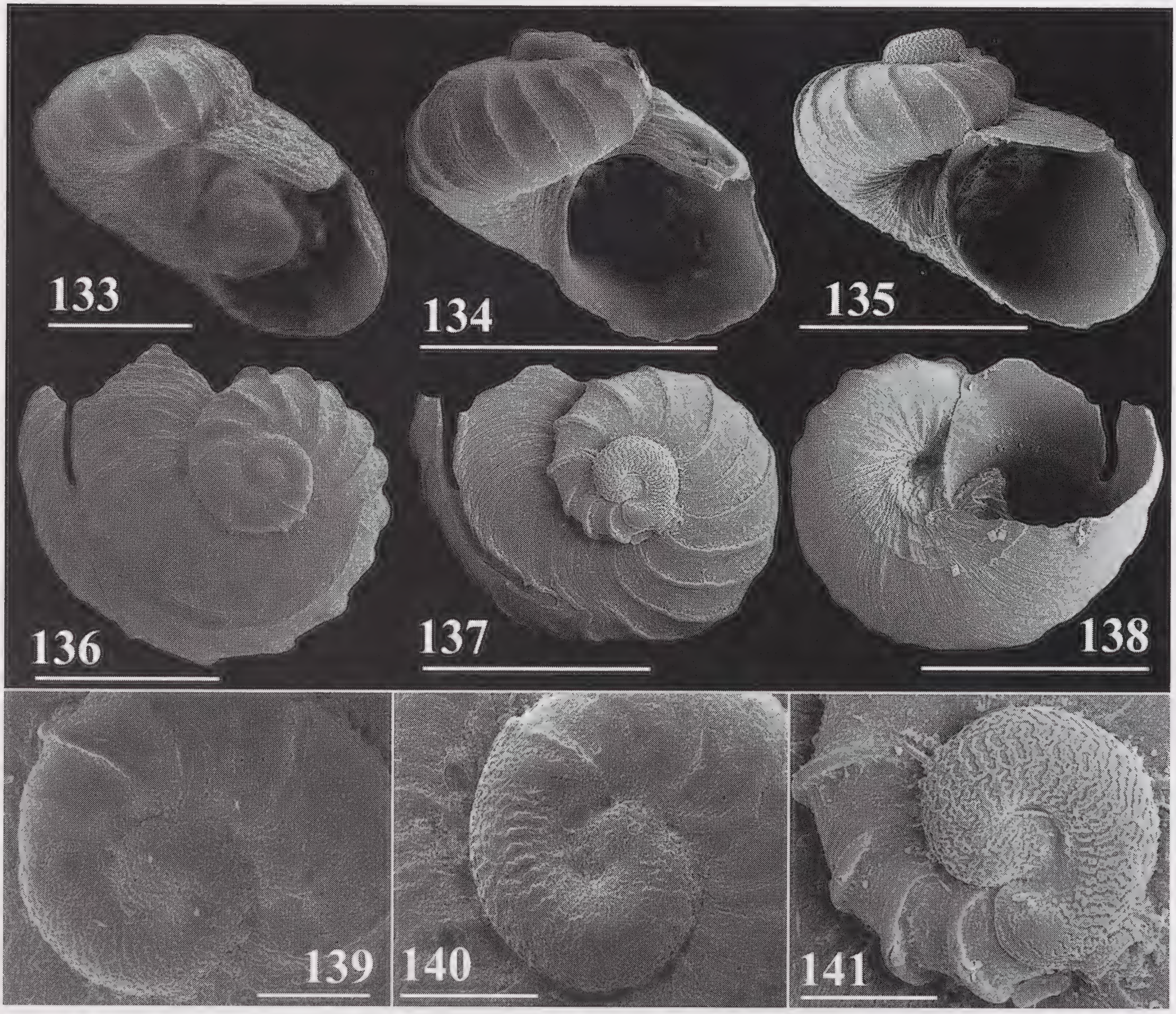
Petermannensis (L.): named for its provenance, the Petermann Island; *timora*: *Timoros* (G.) for honoured; *medioplicata*: *Medio-* (L.) for middle + *-plicata* (L.) for folded, referring to only half of the shell bearing axial ribs.

Diagnosis

The low shell profile with widely spaced and strong axial ribs that extend only over the peripheral portion of the shell distinguish this species from any other known *Scissurella*.

Description

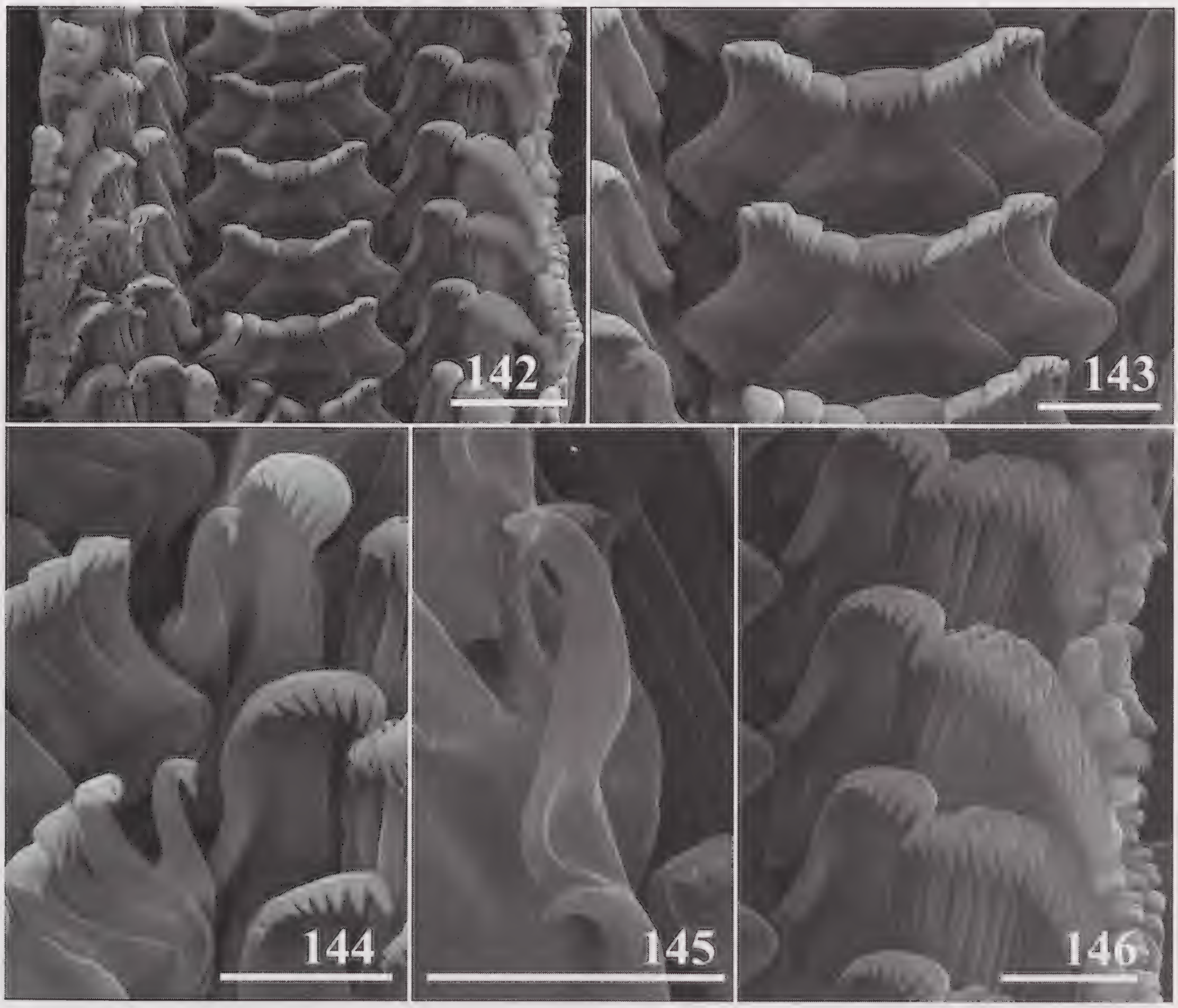
Shell medium size (up to 1.3 mm in diameter), trochiform depressed (Figs. 124–126, 133–135). Protoconch of one whorl, 184–193 µm in diameter, with strong, wide, flat zig-zag



FIGS. 133–141. *Scissurella petermannensis*. FIG. 133: Lectotype of *Scissurella medioplicata* (ZMB-Moll 109.047); FIGS. 134, 136, 139–140: Specimens from 54°51'S, 68°19'W, 5–10 m (MLP 12081); FIGS. 135, 138–139, 141: Specimen from 54°38'S, 158°55'E, Green Gorge, Macquarie Island (AMS C.376623); FIGS. 133–135: Apertural view; FIGS. 136–137: Apical view; FIG. 138: Umbilical view; FIGS. 139–141: Protoconch. Scale bars FIGS. 133–138 = 500 µm; FIGS. 139–141 = 100 µm.

axial ribs, somewhat anastomozing; apertural margin convex, without apertural varix (Figs. 139–141). Teleoconch I of 1 to 1 1/8 whorls, with 10–15 strong, rounded to slightly lamellate axial ribs; axial ornamentation starting sometimes at suture, sometimes at level of selenizone or slightly below periphery (Figs. 130–132, 136–137). Teleoconch II of up to 2/3 whorls. Shoulder slightly convex, overall smooth, only sculptured with irregular growth lines, diminishing in prominence with growth (Figs. 124–126, 133–135). Base with axial ribs and growth lines; occasionally three spiral cords surrounding umbilicus. Umbilicus narrow, deep, with sharp edge transition to base; walls smooth (Figs. 127–129, 138). Selenizone above periphery, keels moderately elevated; slit open, margins parallel (Figs. 124–126, 133–135). Operculum circular with central nucleus.

Radula (Figs. 142–146): Rachidian tooth triangular at base; cusp nearly straight, with central denticle rounded at the tip; 4 narrower, sharper denticles on each side. Lateral teeth 1–3 similar; cusp with four to five denticles on outer margin. Lateral tooth 4 reduced, with one central denticle on cusp, two smaller denticles on outer side. Lateral tooth 5 wider, larger than other lateral teeth, with 8 strong denticles on inner margin, rounded at tip; outer margin smooth. Radular interlock of central field moderate. Inner marginal teeth with main terminal denticle, and three to four denticles on both inner and outer margins; outer mar-



FIGS. 142–146. *Scissurella petermannensis*: radula of one paralectotype of *Scissurella timora* (NMW 1955.158.091). FIG. 142: General view; FIG. 143: Detail of central and lateral teeth 13; FIG. 144: Detail of lateral teeth 4 and 5; FIG. 145: Detail of lateral teeth 4; FIG. 146: Detail of marginal teeth. Scale bars FIG. 142 = 20 µm; FIGS. 143–146 = 10 µm.

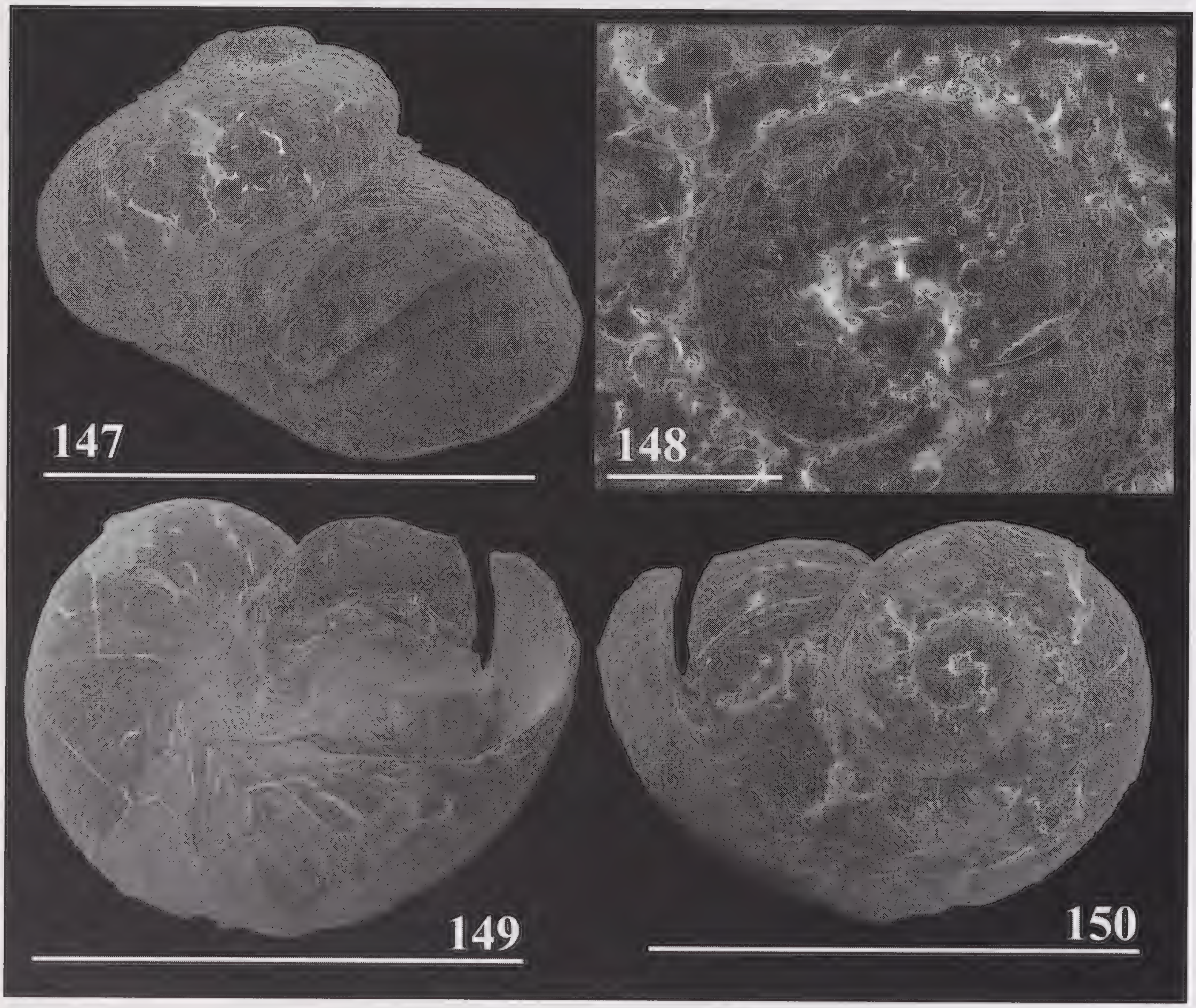
ginal teeth spoon shaped, with narrow but long denticles.

Remarks

Scissurella timora and *S. medioplicata* are here recognized as synonyms of *S. petermannensis*. As these taxa are not well known, we designate here lectotypes for *S. timora* (Figs. 125, 128, 131) and *S. medioplicata* (Fig. 133) with the express taxonomic purpose of taxon stabilization.

The holotype of *Scissurella petermannensis* is a juvenile specimen with only the very beginning of the open slit. The typical descending apertural margin on the last 0.125 whorls are only seen in the type specimens of *S. timora* (Figs. 125, 128, 131). Althought the lectotype and paralectotype of *S. timora* are very eroded, the general shell morphology and ornamentation of these specimens do not differ from that present in the holotype of *S. petermannensis*. The holotype of *Scissurella medioplicata* also agrees in general shell outline with the type specimens of *S. petermannensis* and *S. timora*; due to this fact, this species name is also considered a synonymous. However, it should be noted that the holotype of *S. medioplicata* has more evident axial ribs, but this difference seems to be originated in the better state of preservation of the material, not so eroded as the type specimens of *S. petermannensis* and *S. timora*. In fact, the additional material here studied also show well developed axial ribs.

Cantera & Arnaud (1985) indicated *Scissurella medioplicata* as a synonym of *S. supraplicata*. However, on the basis of the present study, it is clear that the two species



FIGS. 147–150. *Scissurella obliqua*. Lectotype (here designated) (BMNH 1887.2.9.406). FIG. 147: Apertural view; FIG. 148: Protoconch; FIG. 149: Umbilical view; FIG. 150: Apical view. Scale bars FIGS. 147, 149–150 = 1 mm; FIG. 148 = 100 µm.

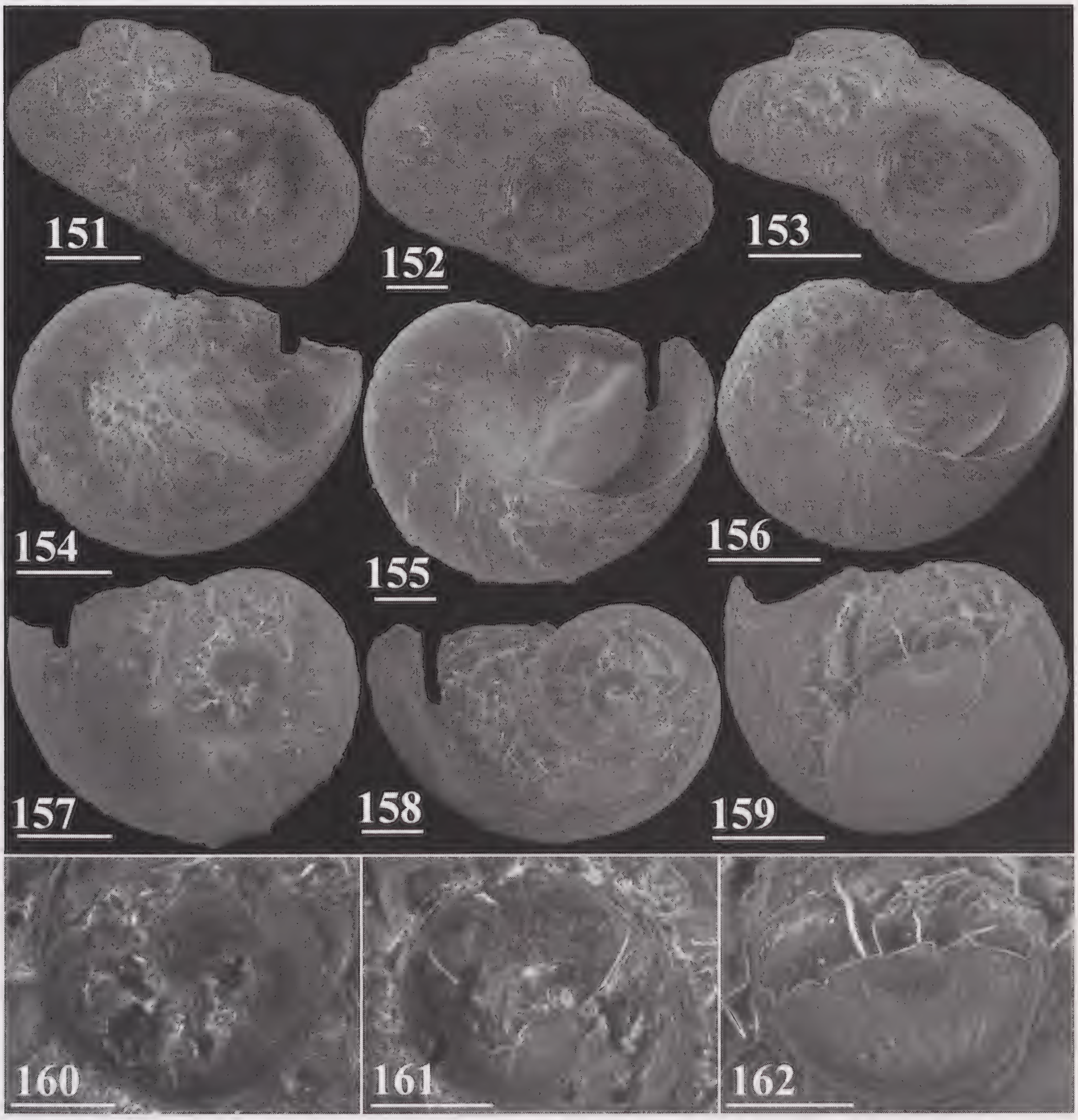
are distinct. In *S. supraplicata* the shell is as wide as height, whereas *S. medioplicata* (a synonymy of *S. petermannensis*) has a depressed, laterally expanded shell.

Castellanos (1980) reported *Scissurella petermannensis* (as *S. timora*) from Malvinas Islands and Strait of Magellan. However, these specimens could not be found in the collections of the MLP or MACN, where other material studied by that author is housed.

The radula of *Scissurella petermannensis* showed the lateral teeth 1–3 with four to five denticles, whereas scissurellids usually has three denticles. Whether this character may be of phylogenetic significance remains to be investigated.

Scissurella obliqua Watson, 1886
Figures 123, 147–162

Scissurella obliqua Watson, 1886: 116, pl. 8, fig. 5; Pilsbry, 1890: 58, pl. 58, figs. 20–21; Thiele, 1912b: 10–11, pl. 1, figs. 22–23; Powell, 1960: 126.
Scissurona? obliqua: Lozouet, 1986: 114.
Incisura? obliqua: Geiger, 2003: 75.



FIGS. 151–162. *Scissurella obliqua*. Three paralectotypes (BMNH 1887.2.9.408). FIGS. 151–153: Apertural view; FIGS. 154–156: Umbilical view; FIGS. 157–159: Apical view; FIGS. 160–162: Protoconch. Scale bars FIGS. 151–159 = 200 μm; FIGS. 160–162 = 100 μm.

Misidentifications:
Scissurella obliqua: Geiger, 2003: fig. 16E [is *Scissurella clathrata*].

Type Locality

Royal Sound, Kerguelen Islands [49°28'S, 70°18'E], shore.

Material Examined

Lectotype (here designated, BMNH 1887.2.9.406) and 6 paralectotypes (BMNH 1887.2.9.408).

Distribution

Known only from the type locality (Fig. 123).

Etymology

Based on the "obliquely globose" shell, as originally described.

Diagnosis

A scissurellid with nearly smooth shell surface, open slit, and shallow umbilicus.

Description

Shell of medium size (up to 1.1 mm in diameter), trochiform depressed, globose; spire small, low (Figs. 147, 151–153). Protoconch of one whorl, 223–256 µm in diameter, with strong, wide, flat zig-zag axials, somewhat anastomizing; apertural margin convex, without apertural varix (Figs. 148, 160–162). Teleoconch I of 1 1/6 to 1 1/4 nearly smooth whorls, only sculptured with low growth marks (Figs. 150, 157–159). Teleoconch II of up to 1/2 whorls. Shoulder somewhat convex, smooth, with minor growth marks; base continuous with umbilical cavity, nearly smooth, with minor growth marks. Aperture rounded-oblong, roof overhanging. Selenizone above periphery, keels very low, moderately strong, with irregular growth lunules; slit with low keels, open, with parallel margins (Figs. 147, 151–153). Umbilicus moderately wide, shallow, not covered by callus (Figs. 149, 154–156). Operculum round, multispiral with central nucleus, covering approximately 80% of aperture. Animal unknown.

Remarks

We select here the best specimen of the type series as the lectotype (Figs. 147–150) with the express taxonomic purpose of taxon stabilization.

Scissurella obliqua is most similar to ***S. malvinarum*** n. sp. (see remarks under that species). *Scissurella obliqua* also resembles *Ariella subantarctica* in general shell outline, but the two species differ in the morphology of the slit: open in *S. obliqua* and closed in *A. subantarctica*. In addition, in *S. obliqua* the umbilicus is open, whereas in *A. subantarctica* it is closed by a strong parietal callus, even in the juveniles. Furthermore, *A. subantarctica* exhibits numerous narrow spiral bands on the last whorl, which are absent in *S. obliqua*.

Scissurella malvinarum n. sp.

Figures 123, 163–174

Type Locality

52°25'S, 59°03'W, Sea Lion Island, Malvinas [Falkland] Islands.

Type Material

Holotype (NMSZ 2006110) and 15 paratypes (NMSZ 1992167.60101) from type locality.

Distribution

Known only from the type locality (Fig. 123).

Etymology

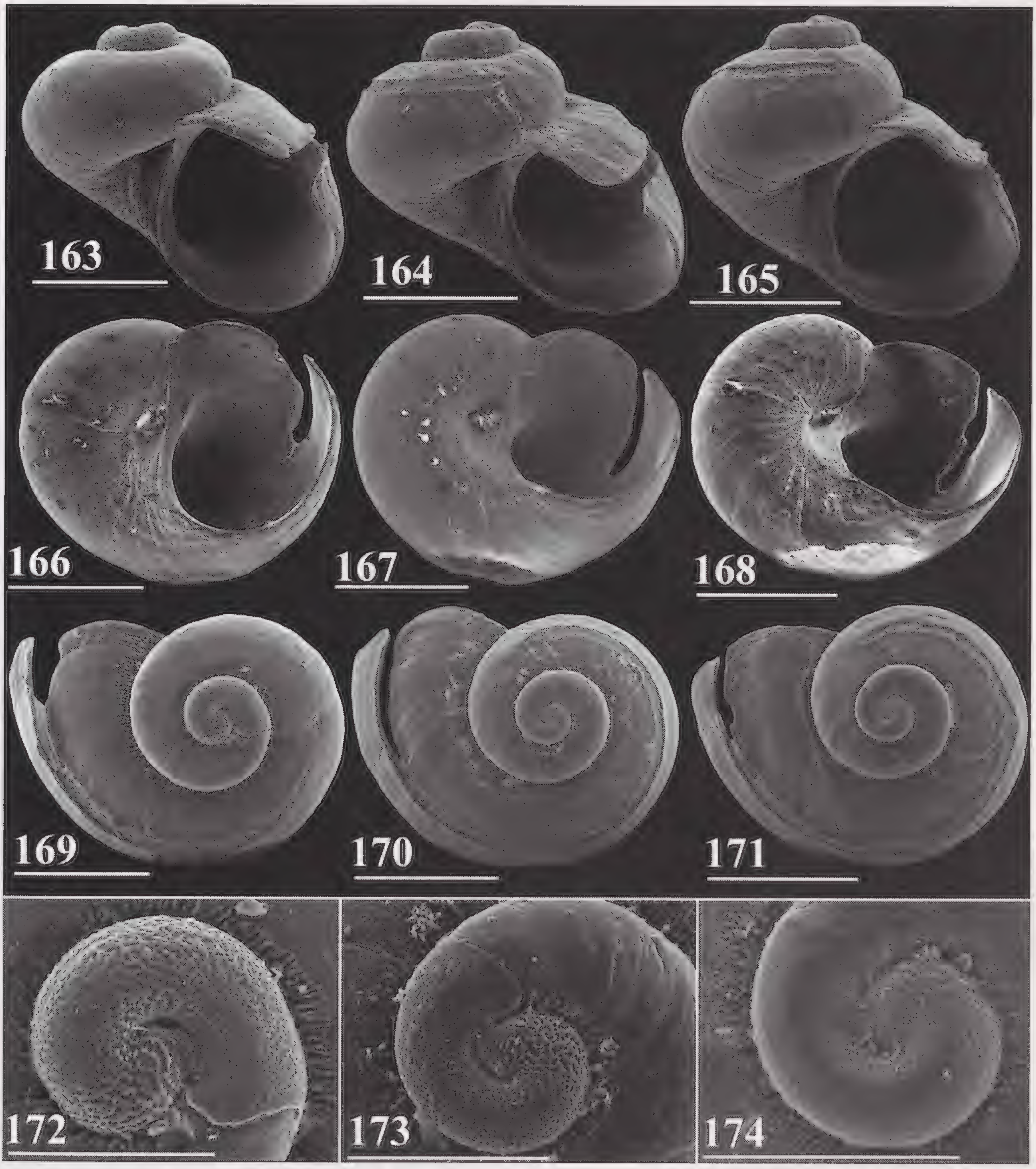
Named after the type locality, the Malvinas Islands.

Diagnosis

A scissurellid with a deep umbilicus surrounded by 2–6 spiral cords, a mesh-like pattern as protoconch ornamentation, and open slit.

Description

Shell of medium size (up to 1.2 mm in diameter), trochiform depressed, globose, low spired (Figs. 163–165). Protoconch of one whorl, 205–258 µm in diameter, scultured with strong, flat irregular cords, forming a mesh-



FIGS. 163–174. ***Scissurella malvinarum***. FIGS. 163, 166, 169, 172: Holotype (NMSZ 2006110); FIGS. 164–165, 167–168, 170–171, 173–174: Paratypes (NMSZ 1992167.60101); FIGS. 163–165: Apertural view; FIGS. 166–168: Umbilical view; FIGS. 169–171: Apical view; FIGS. 172–174: Protoconch. Scale bars FIGS. 163–171 = 500 µm; FIGS. 172–174 = 200 µm.

like pattern; apertural margin convex, without apertural varix (Figs. 172–174). Teleoconch I of one whorl; teleoconch II of up to one whorl (Figs. 169–171). Shell surface sculptured with low growth marks and 2–6 flat spiral cords surrounding the umbilicus. Umbilicus wide, deep (Figs. 166–168). Base continuous with umbilical cavity. Aperture rounded oblong, roof overhanging. Selenizone above periphery, keels very low, with irregular growth lunules; slit open, with parallel margins (Figs. 163–165). Animal unknown.

Remarks

Scissurella malvinarum is most similar to *S. obliqua* from which it differs in having a deeper umbilicus and selenizone more displaced towards periphery. In addition, ***S. malvinarum*** shows spiral cords surrounding the umbilicus, which are completely absent in *S. obliqua*. Furthermore, the protoconch ornamentation of ***S. malvinarum*** shows a mesh-like pattern, while anastomozing zig-zag axials are present in *S. obliqua*. Another similar species is *Ariella subantarctica*, which differs from ***Scissurella malvinarum*** in having a close foramen, the umbilicus filled by a callus, and more developed shell sculptured. ***Scissurella malvinarum*** is a mature *Scissurella* and not a juvenile *Sinezona* with open slit. The last quarter of the whorl is descending while the margins of the slit do not converge (Fig. 164), a clear sign of maturity as discussed by Geiger (2003, 2006a).

Scissurella supraplicata Smith, 1875
Figures 123, 175–178

Scissurella supraplicata Smith E. A., 1875: 72 (not illustrated); Crosse, 1877: 10; Smith, 1879: 176, pl. 9, fig. 5, 5a; Paetel, 1888: 289; Pilsbry, 1890: 54–55, pl. 58, figs. 27–28; Thiele, 1912b: 10, pl. 1, figs. 20–21; Melvill & Standen, 1912: 345; Thiele, 1925: 42; Powell, 1957: 124; Powell, 1960: 126; Cantera & Arnaud, 1985: 32; Geiger, 2003: 78.
Scissurella (?) supraplicata: Lozouet, 1986: 108.
Misidentifications:
Scissurella supraplicata: Melvill and Standen, 1912: 345 [is *Anatoma conica*].
Schizotrochus supraplicata [*sic*]: Carcelles & Williamson, 1951: 251 [is *A. conica*].
Schizotrochus supraplicatus: Carcelles, 1953: 162 [is *A. conica*].
Scissurella supraplicata: Ageitos de Castellanos & Landoni, 1988: 8 [is *A. conica*].

Type Locality

Swain's Bay, Kerguelen Island [49°42'S, 69°57'E] (OD).

Material Examined

Holotype (BMNH. 1876.8.28.33).

Distribution

Known only from the type locality (Fig. 123).

Etymology

Supra-: (L.) for above, on top; *-plicata*: (L.) for folded.

Diagnosis

Shell inflated, with strong axial ribs on teleoconch I, slit open, and narrow umbilicus.

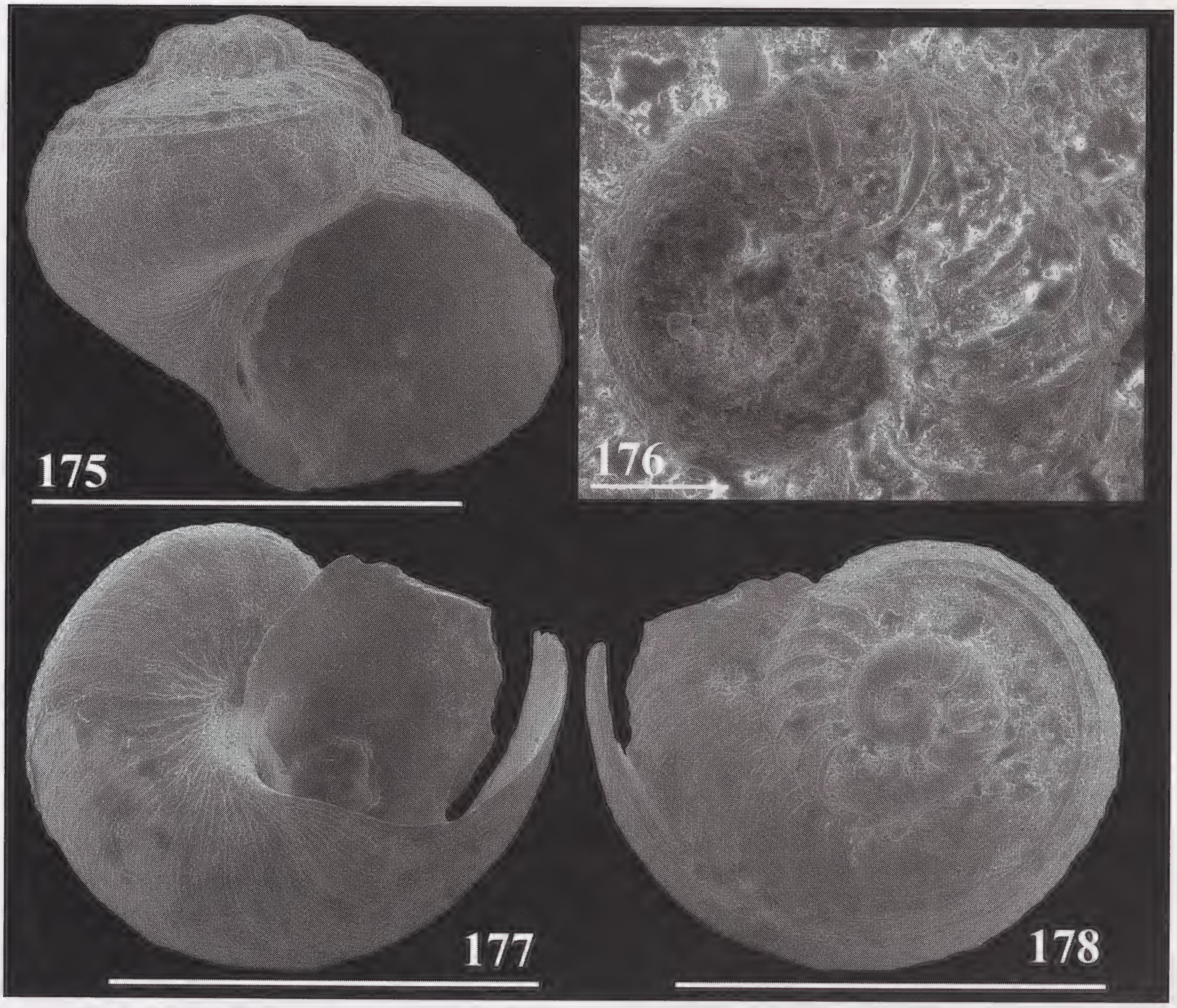
Description

Shell medium in size (1.25 mm in diameter in the holotype), trochiform, inflated-bulbous (Fig. 175). Protoconch of one whorl, about 220 µm in diameter; apertural margin convex, without varix (Fig. 176); protoconch ornamentation unknown (very eroded in only known specimen). Teleoconch I of one whorl, sculptured with 17 strong axial ribs (Fig. 178). Teleoconch II of about one whorl, with convex shoulder and inflated base, sculptured with axial growth folds; axials making more prominent toward umbilicus (Figs. 175, 177). Selenizone above periphery, surrounded by low keels, having low growth marks; slit open, with parallel margins (Fig. 175). Umbilicus narrow (Fig. 177). Aperture rounded. Animal unknown.

Remarks

Scissurella supraplicata resembles *S. clathrata* in general shell outline, but these species differ in shell ornamentation: while the former shows strong axial sculpture (particularly in teleoconch I), the latter has a delicate cancellate pattern. In addition, the umbilicus is narrower in *S. supraplicata* than in *S. clathrata*. Another similar species is *Sinezona mouchezi*, which differs from *Scissurella supraplicata* by having a closed foramen, a more laterally expanded shell, and wider umbilicus.

Cantera & Arnaud (1985) indicated *Scissurella medioplicata* as a synonym of *Scissurella supraplicata*, an opinion not shared here: *S. medioplicata* [a junior synonymy of *S. petermannensis*] has a more laterally expanded shell, with stronger axial ribs and a deeper umbilicus than *S. supraplicata*.



FIGS. 175–178. *Scissurella supraplicata*. Holotype (BMNH 1876.8.28.33). FIG. 175: Apertural view; FIG. 176: Protoconch; FIG. 177: Umbilical view; FIG. 178: Apical view. Scale bars FIGS. 175, 177–178 = 1 mm; FIG. 176 = 100 µm.

Carcelles & Williamson (1951) and Ageitos de Castellanos & Landoni (1988) included *Scissurella supraplicata* in the list of species from the Magellan Region, based on the record of Melvill & Standen (1912), which, as indicated above, does not correspond to this species but to *Anatoma conica*. The same source of error seems to be the origin of the inclusion of *S. supraplicata* in the list of species by Carcelles (1953).

Scissurella dalli Bartsch, 1903, *nomen dubium*

Scissurella dalli Bartsch, 1903: 90–91; Thiele, 1912b: 8; Carcelles, 1950: 50; Ageitos de Castellanos & Landoni, 1988: 9; Geiger, 2003: 76.

Schizotrochus dalli: Carcelles & Williamson, 1951: 251.

Type Locality

Gulf of St. George, Patagonia [~ 46°S, 66°W, Argentina] (OD).

Etymology

Named after William Healy Dall (1845–1927).

Remarks

The species was not illustrated by Barsch (1903), and has never been figured elsewhere. Bartsch's description lacks any discernible detail necessary to evaluate the species. The type material is missing from USNM (Geiger, pers. obs. 2/2004) where it was originally deposited (fide Bartsch, 1903). The generic placement of this species is tentative; Bartsch noted the selenizone "near periphery", which applies to *Scissurella*, while in *Anatoma* the selenizone is at the periphery; *Anatoma* and *Scissurella* were not used as distinct genera in the early 20th century. Whether Bartsch's species was based on an immature specimen of *Sinezona* remains open.

Ariella Bandel, 1998

Type Species (OD): *Ariella haliotimorpha* Bandel, 1998.

Diagnosis

Shell approximately 1 mm, trochiform depressed, protoconch with axial sculpture; foramen above periphery, no selenizone. More comprehensive diagnoses and discussions have been provided elsewhere (Geiger, 2003, 2006a; Geiger & Jansen, 2004b).

Remarks

Scissurella and *Sinezona* show a selenizone; *Satondella* has the walls of the foramen forming a chimney; *Scissurella* also has an open slit. The validity of the genus is debatable, as discussed by Geiger (2003); we conservatively retain it here.

Ariella subantarctica (Hedley, 1916)
Figures 179, 180–196

Schismope subantarctica Hedley, 1916b: 36, pl. 5, figs. 54–55; Finlay, 1927: 341.
Sinezona subantarctica: Powell, 1955: 46; Powell, 1960: 126; Powell, 1979: 35; Spencer & Willan, 1995: 13.
Scissurella obliqua Watson: Cantera & Arnaud, 1985: 31 [non Watson, 1886].
Sinezona? subantarctica: Lozouet, 1986: 111.
? *Sinezona* sp.: Branch et al., 1991: 47, 58, 62.
Ariella subantarctica: Geiger, 2003: 75, fig. 15g.

Type Locality

Aerial Cove, Macquarie Island [54°29'S, 158°57'E] (OD).

Material Examined

Macquarie Islands: 54°30'S, 158°53'E, Handspike Point (18 shs, AMS C.376612); Aerial Cove: 54°29'S, 158°57'E, 5 m (6 sps, AMS C.376621); 5 m (2 sps, NMNZ M.152863). Crozet Islands: 46°25'S, 52°12'W, South Marin Bay, in clumps of Durvillea (2 shs, MNHN). Southern Chile: 50°11'S, 74°48'W, Bahía Tom, 15 m (2 shs, LACM 73-72); 46°35'S, 75°30'W, Bahía San Andres, 1 m (11 sps, LACM 73-73). Southern Argentina: 54°48'S, 65°16'W, Bahía Buen Suceso, 0 m (1 sh, LACM 71-270).

Distribution

Macquarie Island, Crozet Islands, Magellan Region. 0–15 m (living specimens: 1–5 m) (Fig. 179).

Etymology

Referring to its provenance, a subantarctic island.

Diagnosis

Shell depressed, with prominent umbilical callus, closed foramen, no obvious selenizone, and sculptured with axial ribs and spiral cords.

Description

Shell medium size (up to 1.3 mm in diameter), trochiform-depressed (Figs. 182–184). Protoconch of 1 1/8 to 1 1/2 whorls, 198–202 µm in diameter, with strong irregular flattened anastomozing axial ribs; apertural margin slightly convex, with varix connected to embryonic cap (Figs. 191–193). Teleoconch I of 1 1/6–1 5/8 whorls, sculptured with about 40 axial ribs of variable degree of development, and delicate spiral sculpture (Figs. 180, 188–190). Teleoconch II of 1/8 whorl. Shoulder slightly convex; axial sculpture as on teleoconch I, additionally one dozen spiral cords increasing in width towards apertural margin. Base with 8–9 spiral cords; axial sculpture weak (Figs. 181–187). Umbilicus open in juveniles, closed by callus in adult specimens (Figs. 185–187). Selenizone extremely short or absent; foramen lenticular, with very low keels, continued anteriorly by raphe (Figs. 180–181, 185–190). Aperture broadly rounded, oblique to shell axis, roof overhang-

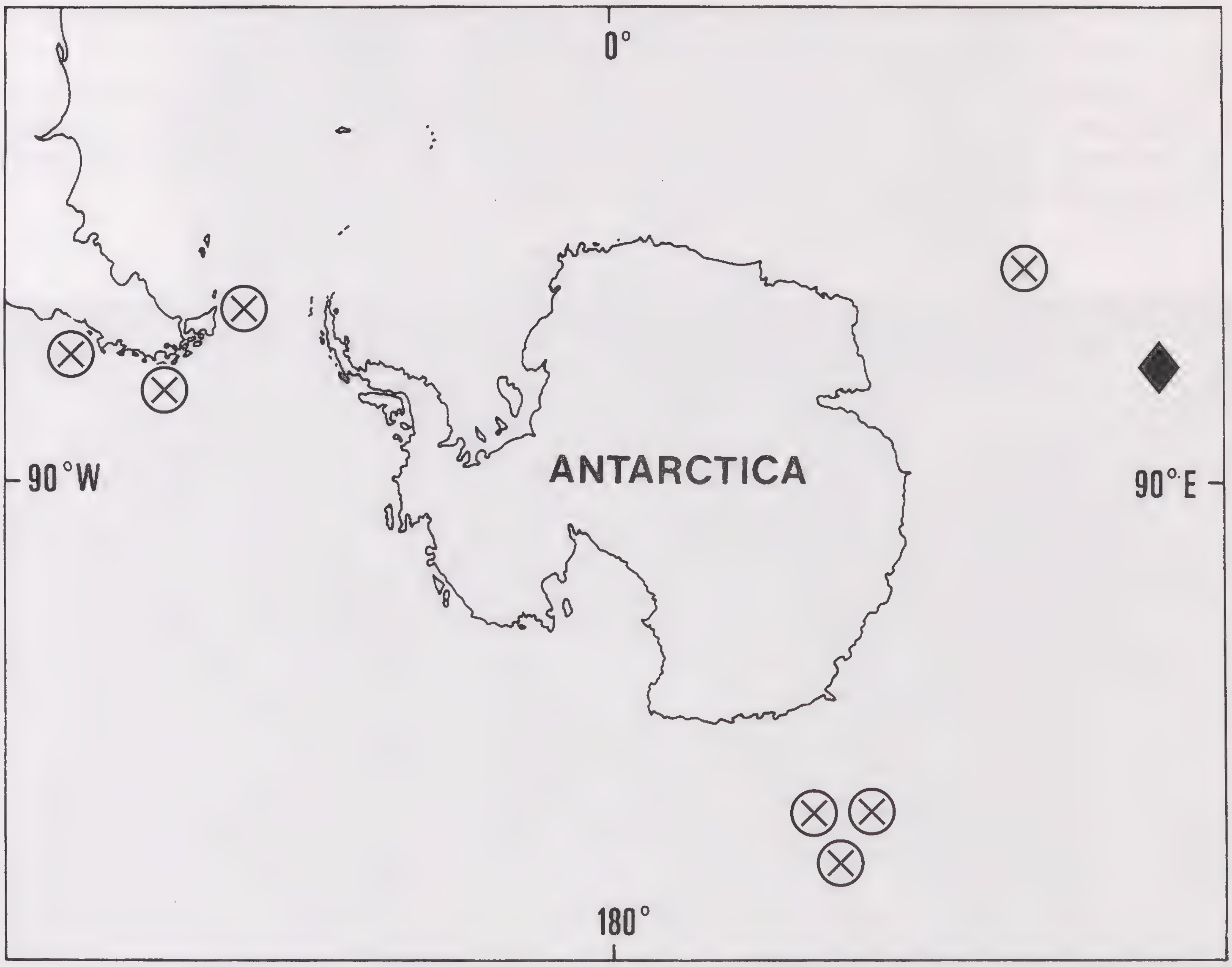
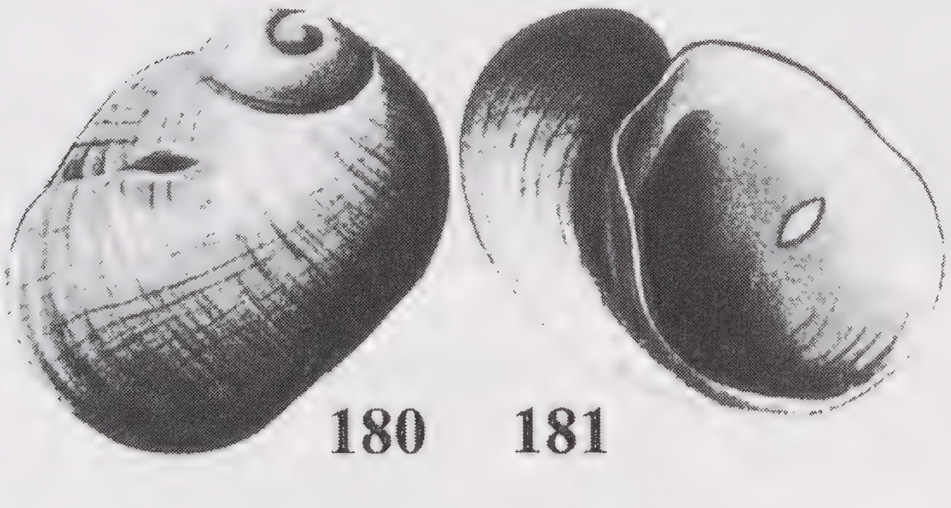


FIG. 179. Distribution of *Ariella subantarctica* (⊗) and *Sinezona mouchezi* (◆).

ing; umbilical callus as extension of peristome. Operculum very thin, multispiral, with central nucleus, covering approximately 60% of aperture width.

Radula (Figs. 194–196): Rachidian tooth with broadly triangular base; cusp nearly straight at lateral edges, V-shaped distally, with one main rounded denticle and 2–3 smaller denticles at each side, reducing size towards base.



FIGS. 180–181. *Ariella subantarctica*. Original figures by Hedley (1916b).

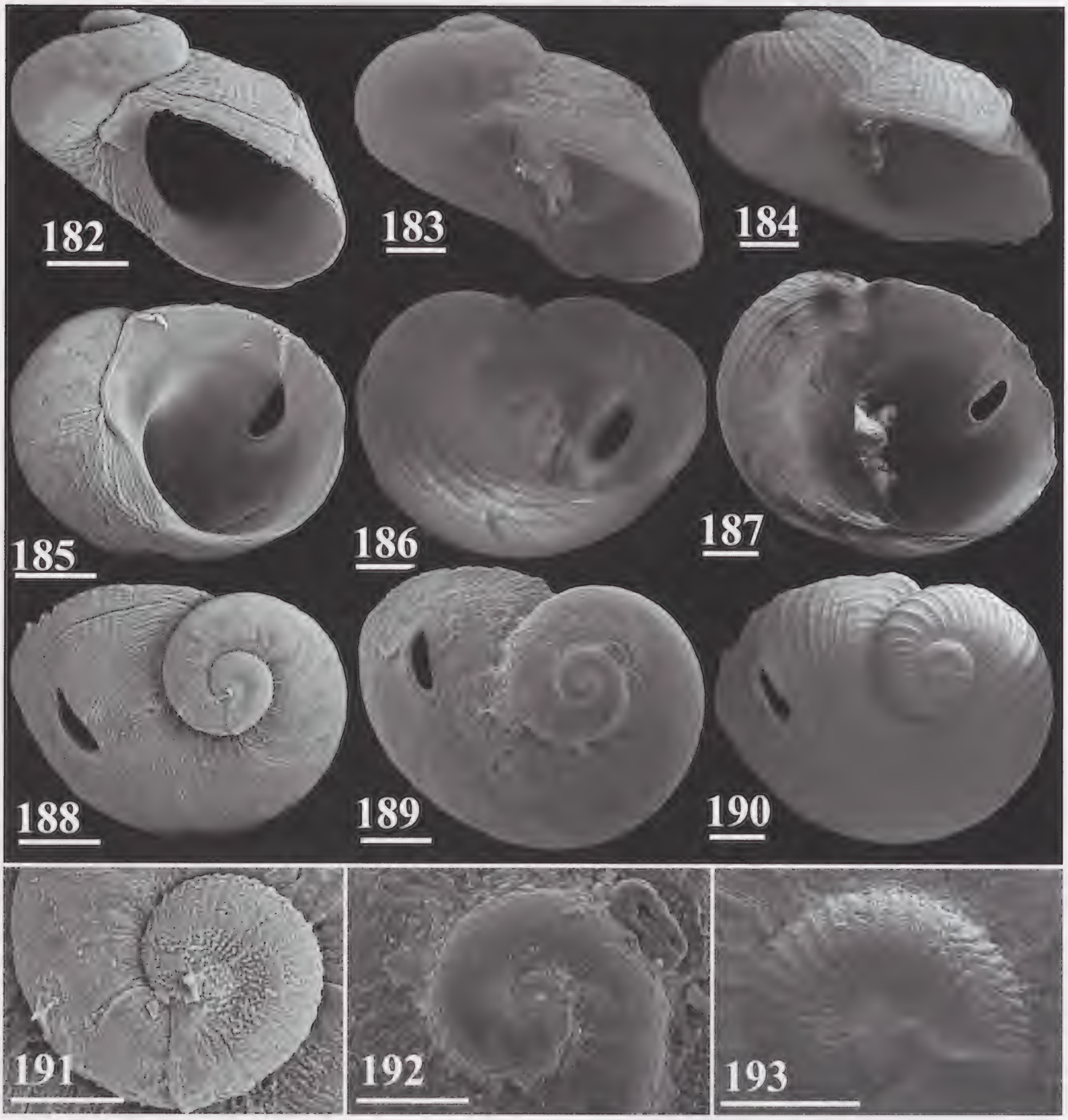
Lateral teeth 1–3 similar, with three strong, sharp denticles on outer edge. Lateral tooth 4 reduced, with one small point on either side of main denticle. Lateral tooth 5 larger and wider than other laterals; cusp smooth on outer edge, with 7 denticles on inner edge. Radular interlock of central field moderate. Inner marginal teeth with one main central denticle, two denticles on inner edge of cusp, and three denticles on outer edge. Outer marginals spoon-shaped.

Remarks

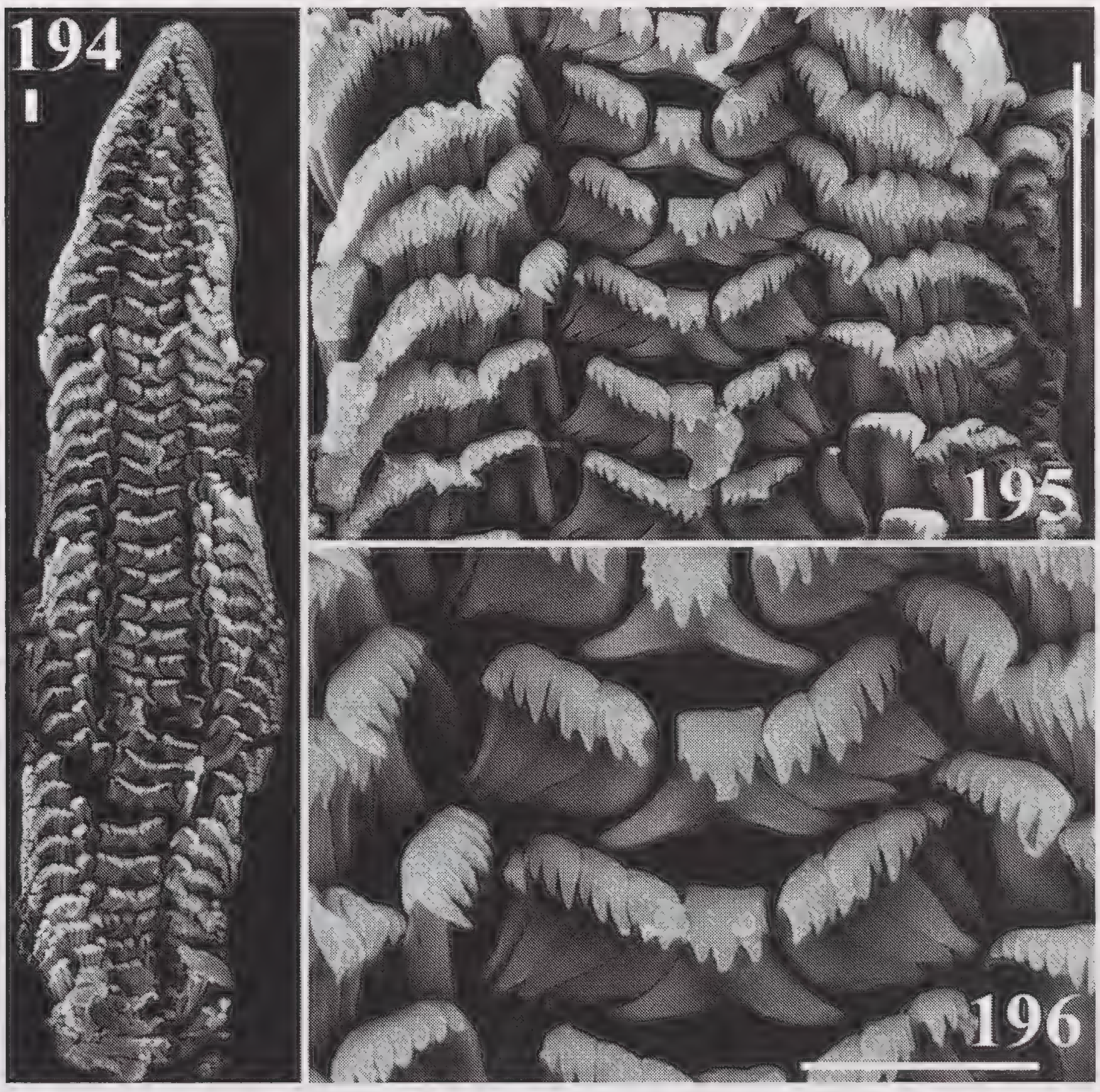
According to Powell (1955), the holotype of *Ariella subantarctica* was originally housed at the AMS, but this material was not found during a visit to this museum in 2001 (Geiger, pers. obs.). However, the identity of this species could be confirmed by the examination of several topotypical specimens.

Ariella subantarctica resembles in general shell outline *Scissurella obliqua*, from which it differs by having a close slit forming a foramen, well-developed axial and spiral sculpture, and the umbilicus covered by a strong callus. The other species from the area with a closed slit is *Sinezona mouchezi*, but the later has a long selenizone (which is absent in *A. subantarctica*), a higher spire, more distinct axial ribs on the teleoconch, and fewer straight axials on the protoconch.

The re-examination of the material reported by Cantera & Arnaud (1985) as *Scissurella obliqua* (MNHN) showed that it is referrable to *Ariella subantarctica*.



FIGS. 182–193. *Ariella subantarctica*. FIGS. 182, 185, 188, 191: Specimen from 54°30'S, 158°53'E, Handspike Point, Macquarie Islands (AMS C.376612); FIGS. 183, 186, 189, 192: Specimen from Aerial Cove, Macquarie Island, 5 m (NMNZ M.152863); FIGS. 184, 187, 190, 193: Specimen from 46°35'S, 75°30'W, Bahía San Andres, 1 m (LACM 73-73); FIGS. 182–184: Apertural view; FIGS. 185–187: Umbilical view; FIGS. 188–190: Apical view; FIGS. 191–193: Protoconch. Scale bars FIGS. 182–190 = 200 µm; FIGS. 191–193 = 100 µm.



FIGS. 194–196. *Ariella subantarctica*. Radula of a specimen from 54°29'S, 158°57'E, Aerial Cove, 5 m (AMS C.376621); FIG. 194: Entire radula; FIG. 195: Full width of radular ribbon; FIG. 196: Central field enlarged. Scale bars = 10 µm.

Genus: *Sinezona* Finlay, 1926

Type Species (OD): *Schismope brevis* Hedley, 1904.

Diagnosis

Shell 0.7–3 mm, trochiform, more or less depressed; protoconch usually with axial sculpture, sometimes smooth or with spiral elements; selenizone, foramen above periphery. More comprehensive diagnoses and discussions have been provided elsewhere (Geiger, 2003, 2006a; Geiger & Jansen, 2004b; Marshall, 2002).

Remarks

Scissurella has an open slit, *Ariella* lacks the selenizone, *Satondella* has the foramen forming a chimney.

Sinezona mouchezi (Vélain, 1876)
Figures 179, 197–212

Schismope mouchezi Vélain, 1876: 285; Vélain, 1877: 119–120, pl. 4, figs. 7–8; Paetel, 1888: 289; Pilsbry, 1890: 62, pl. 57, figs. 18–19; Thiele, 1912b: 25, pl. 3, figs. 21–22.

Sinezona mouchezi: Lozouet, 1986: 111; Geiger, 2003: 79.

Type Locality

St. Paul Island [38°44'S, 77°30'E], subtidal to 45 m (OD).

Material Examined

Lectotype (here designated) and 3 paralectotypes (MNHN).

Distribution

Known only from the type locality (Fig. 179).

Etymology

Named after the Commandant Mouchez.

Diagnosis

Shell inflated, sculptured with strong axial ribs, and having a wide umbilicus and large foramen.

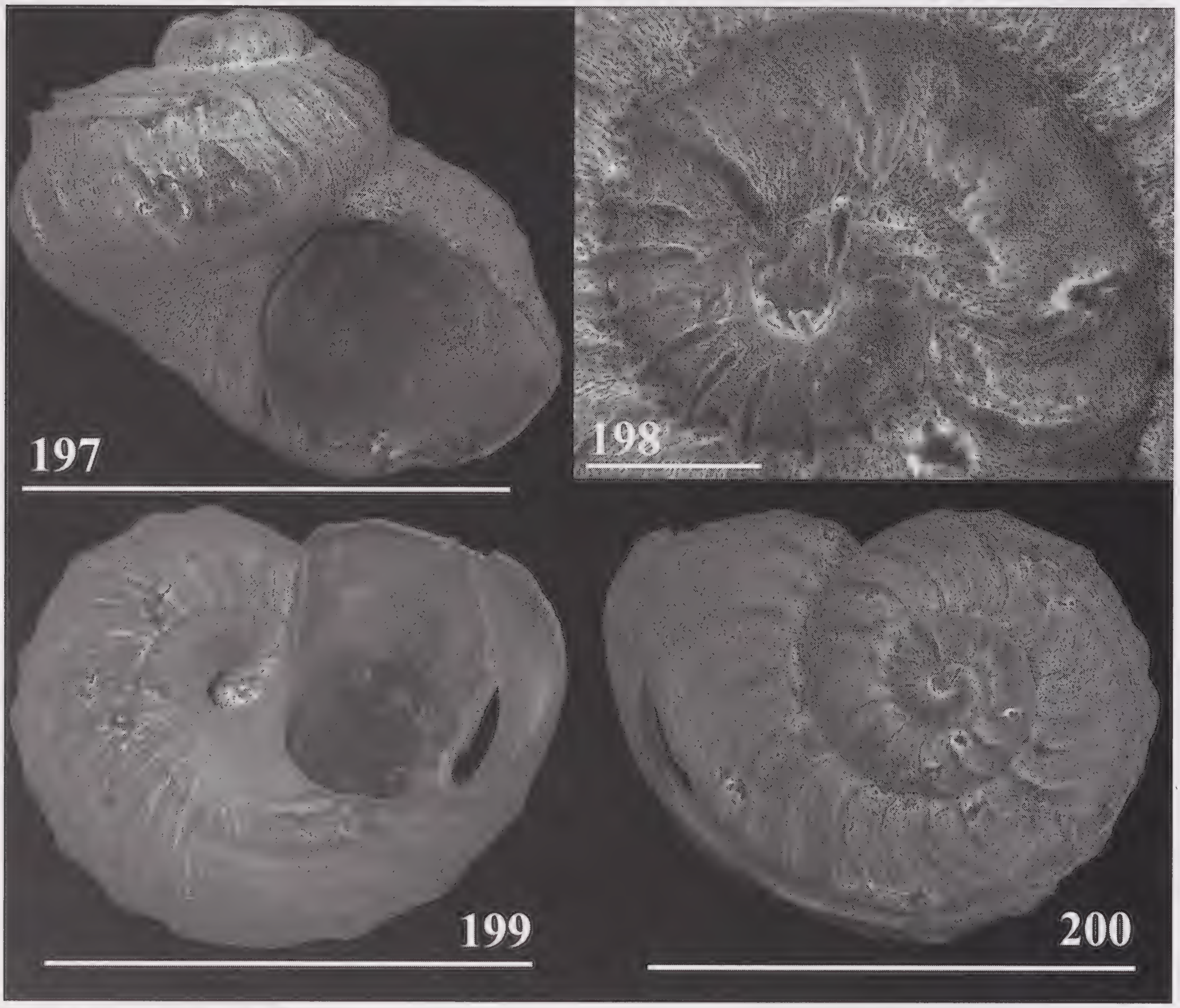
Description

Shell medium in size (up to 2 mm in diameter), trochiform, inflated, last whorl somewhat laterally expanded (Figs. 197, 201–203). Protoconch of one whorl, 193–203 µm in diameter; protoconch sculptured with 13–15 strong axial ribs, distinct varix connected to embryonic cap (Figs. 198, 210–212). Teleoconch I of 1 1/8 whorls, sculptured with 13–14 strong axial ribs, 6 narrower spiral threads (Figs. 200, 207–209). Teleoconch II of up to 7/8 whorls, with convex shoulder, inflated base, sculptured with strong axial ribs, and spiral threads; 14–16 axial ribs on shoulder, about 13 on base, diminishing in prominence toward umbilicus; spirals regularly distributed, 6–7 on shoulder, 7–8 on base (Figs. 197–199, 201–209). Selenizone above periphery, surrounded by low keels, having low growth marks; slit closed forming foramen (Figs. 200, 207–209). Umbilicus wide (Figs. 199, 204–206). Aperture rounded. Animal unknown.

Remarks

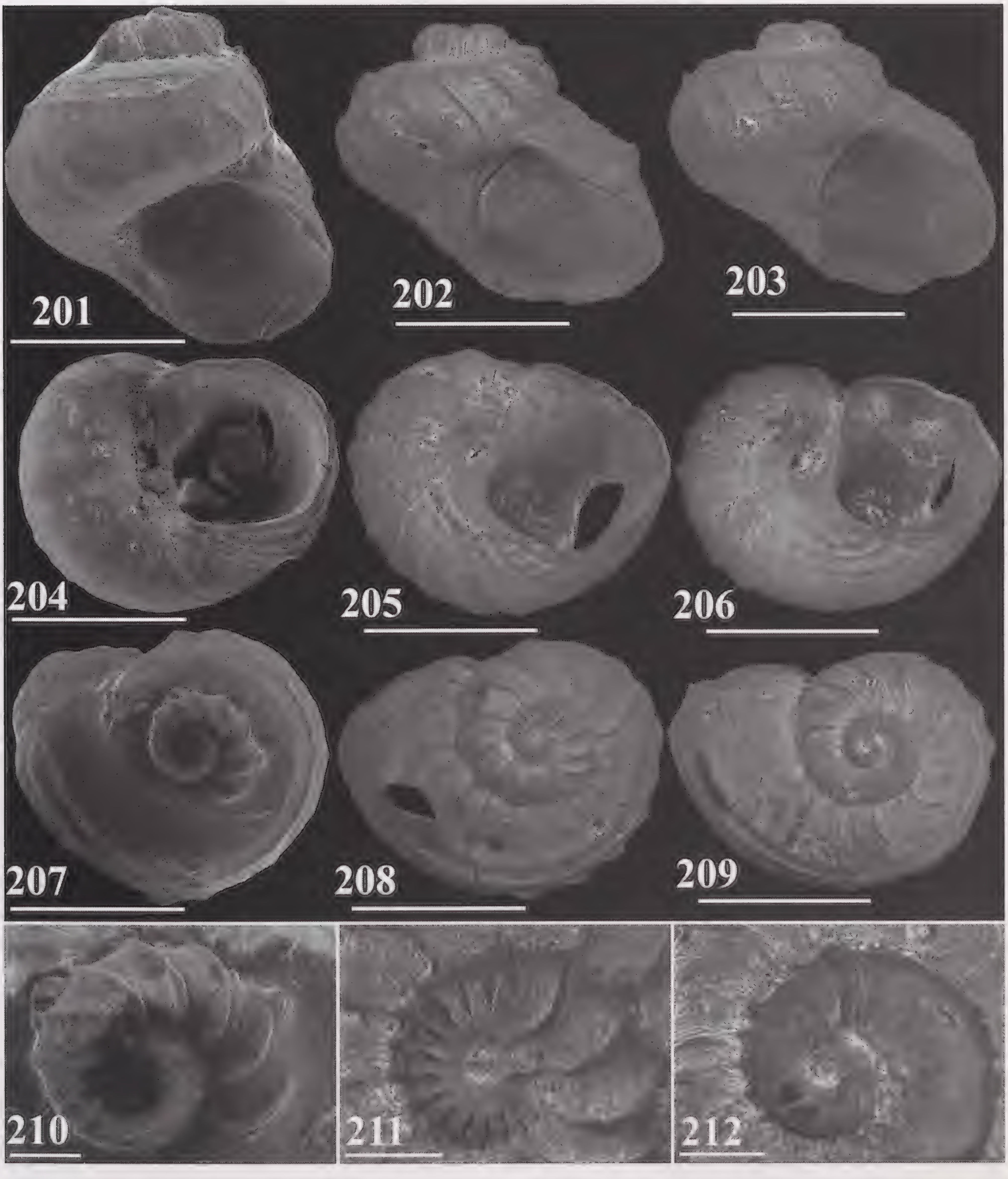
Sinezona mouchezi closely resembles *Scissurella supraplicata* from which it differs by having a close foramen and wider umbilicus.

Among the four specimens that represent the type series of *Sinezona mouchezi* two "varieties" can be recognized: three of the specimens have the above described set of characters; the other specimen has a taller spire, a less laterally expanded shell, and the axial and spiral ornamentation is almost absent on the last whorl; these characteristics are correlated with a larger overall size of teleoconch II. How-



FIGS. 197–200. *Sinezona mouchezi*. Lectotype (here designated). FIG. 197: Apertural view; FIG. 198: Protoconch; FIG. 199: Umbilical view; FIG. 200: Apical view. Scale bars FIGS. 197, 199–200 = 1 mm; FIG. 198 = 100 µm.

ever, the number of protoconch and teleoconch I whorls, and their ornamentation is the same in all the specimens; consequently, we consider these differences as infraspecific variability. None of the above-mentioned specimens correspond to the material figured by Vélain (1877), which has the teleoconch with a larger number of whorls (3 1/4), a taller spire, and a more prominent shell sculpture. Taking into account this fact and that the young specimens of *S. mouchezi* could be confused with *Scissurella supraplicata*, we designate here a lectotype of the former with the express purpose of taxon stabilization.



FIGS. 201–212. *Sinezona mouchezi*. Paralectotypes. FIGS. 201–203: Apertural view; FIGS. 204–206: Umbilical view; FIGS. 207–209: Apical view; FIGS. 210–212: Protoconch. Scale bars FIGS. 201–209 = 500 µm; FIGS. 210–212 = 100 µm.

DISCUSSION

In the present study the identity, taxonomic status, and distribution of 14 of the 15 nominal species of scissurellids and anatomids previously reported from the Antarctic and Sub-Antarctic waters are evaluated; the remaining species (*Scissurella georgica*) was recently and properly described and figured by Davolos & Moolenbeek (2005), and consequently is not redescribed herein. Out of the 15 nominal species known from the area, 11 proved to be valid, another species (*Scissurella dalli*) is a *nomen dubium*, and the remaining three species (*Scissurella eucharista*, *S. timora*, and *S. medioplicata*) proved to be synonyms. The new species were described at the time and were not recognized as mere variants of described species because of the limited material available and the wide geographic distribution of the species. In addition, three other species examined during this study proved to be new to science, and are also described herein: ***Thieleella weddelliana***, ***T. argentinae***, and ***Scissurella malvinarum***.

Many of the taxonomic uncertainties regarding the Sub-Antarctic and Antarctic scissurellid and anatomid species can be accounted for species having been described based on single specimens, often in poor preservational state, and that they were never properly illustrated. It resulted in numerous misidentifications here detected in literature. In the present study, this limitation was overcome by studying large collections from the Southern Ocean and several topotypic specimens, and using SEM for all aspects of the investigation.

New Morphological and Anatomical Observations

The present work has shown some further intrageneric variability with respect to radular morphology. The rachidian tooth of Scissurellidae had considered previously to be diagnostically shaped, with a V-shaped cusp (Geiger, 2003). In *Scissurella clathrata* and *S. petermannensis*, however, the cusp is approximately parallel to the row of teeth, a condition previously reported only from anatomid species. However, the number of denticles present in the cusp continues being an adequate character to separate Scissurellidae and Anatomidae: in scissurellids there is consistently a smaller number of proportionally larger denticles. The absolute size of the denticles is approximately the same in *Scissurella clathrata* and *Thieleella amoena*. The rachidian tooth of *Scissurella clathrata* is approximately 4 µm wide and has five denticles, whereas in *Thieleella amoena* the rachidian tooth is 8 µm wide with 9–11 denticles. On the other hand scissurellid species over a wide size range from *Scissurella kaiserae* Geiger, 2006, of 0.6 mm to *Sinezona plicata* (Hedley, 1899) up to 3 mm in diameter, all have a very similar arrangement of the rachidian tooth with four or five denticles (Geiger & Jansen, 2004b; Geiger, 2006a). Ontogenetic data on the radula in either Scissurellidae or Anatomidae is not available.

The denticles on lateral tooth five are also diagnostic for Scissurellidae and Anatomidae. Scissurellidae have denticles only on the inner margin of the cutting edge; the outer edge is smooth. In contrast, all Anatomidae species investigated thus far have well-developed and narrower denticles on both sides of the cutting edge.

Another diagnostic character is the orientation of the lateral tooth five on the ribbon. This tooth is oriented with the tip pointing to midline of radula or parallel to radular axis in anatomids, whereas it points towards marginals in scissurellids.

In the present study, we provide for the first time information of the jaw plate for an anatomid (*Anatoma euglypta*). The relevance of this character in systematic is still unknown, and should be evaluated after its examination in other species and genera.

The anatomy of scissurellids and anatomids is currently known in only few species (Barret, 1856; Vayssière, 1894; Pelseneer, 1899, 1903; Bourne, 1910; Fretter & Graham, 1962, 1976; Herbert, 1986; Hazprunar, 1989; Strasoldo, 1991). Among the species occurring in Sub-Antarctic and Antarctic waters, some data on the gross anatomy is only available for *Anatoma euglypta* (Pelseneer, 1903). We obtained here some additional information on the anatomy of *A. euglypta* and the first data for *Scissurella clathrata*, *Thieleella amoena*, and ***T. weddelliana***. All these species agree in general morphology with the species from tropical regions and the northern hemisphere (e.g., Herbert, 1986; Strasoldo, 1991).

The present study revealed the existence of some differences in the morphology of cephalic tentacles among the species here examined: they were long and cylindrical in *Anatoma euglypta* and *Thieleella amoena*, long and lanceolated in *Anatoma weddelliana*, and short and foliated in *Scissurella clathrata*; all of them showed papillate tentacles. In other species for which this character was described, these tentacles were always described as cylindrical, and sometimes as lacking of papillae (e.g., *Temnocinclis euripes* McLean, 1989 and *Temnozaga parilis* McLean, 1989: Hazprunar, 1989, althought the affinity of these vent taxa currently classified in Scissurellidae is not adequately resolved).

Another anatomical character that proved to be useful as a diagnostic feature is the coiling pattern of the hindgut. The intestine is very long and forms several loops in all the species here studied, as well as in other species of scissurellids and anatomids for which the anatomy is known. The portion below the roof of the pallial cavity (the "hindgut") shows different patterns in *Anatoma euglypta*, *Thieleella amoena*, ***T.weddelliana***, and *Scissurella clathrata*. Heath (1937) found in the genera *Nucula* and *Acila* (Bivalvia) that there is a correlation between length of intestine and depth at which species live; Sanders & Allen (1973) also noted a relative increase of the hindgut as size of some protobranch bivalves decreases. This seems to apply to the species considered herein: *Scissurella clathrata*, a shallow-water species, has a shorter and less twisted intestine than *Anatoma euglypta*, *Thieleella amoena*, and ***T. weddelliana*** (all of them from deeper waters). However, it should be noted that these differences might be also related with the familiar assignment of these species (Scissurellidae vs. Anatomidae). Consequently, we consider that more detailed studies on this topic are required to confirm this observation.

Geographical Distribution and Ecological Patterns

The studied species showed varied distributional patterns, ranging from species with rather restricted geographic distribution to species widely distributed. Such species as *Anatoma shiraseae*, ***Thieleella weddelliana***, ***T. argentinae***, *Scissurella obliqua*, *S. supraplicata*, *S. georgica*, ***S. malvinarum***, and *Sinezona supraplicata* are known only from their type localities. These apparently restricted geographical ranges seemed to be a result of the limited knowledge from the Southern Ocean. *Scissurella clathrata* and *Anatoma conica*, two species known from many localities in the Magellan Region, may be also considered as belonging to the group of species with relatively restricted distributional ranges. *Ariella subantarctica* occured in two different areas: it was found both in true Sub-Antarctic waters (Macquarie Island, Crozet Island), and in the Magellan Region. A wider distribution was shown by *Scissurella petermannensis*, occuring at the Antarctic Peninsula, the Scotia Arc Islands (South Shetland Islands, South Orkney Islands), the Sub-Antarctic islands (Kerguelen and Macquarie), and the Magellan Region (Tierra del Fuego). Finally, *Thieleella amoena* and *Anatoma euglypta* showed a circumantarctic distribution, the latter also spreading northwards, following Sub-Antarctic waters.

Four of the species present in the Antarctica belong to Anatomidae: *Anatoma euglypta*, *A. shiraseae*, *Thieleella amoena*, and ***T. weddelliana***; it is interesting to note that only one scissurellid species (*Scissurella petermannensis*) is known from this area. However, scissurellids are common in Sub-Antarctic waters, where they are represented by four species. A similar pattern occurs in the higher latitudes of the northern hemisphere: in Europe, scissurellids (*Scissurella costata* d'Orbigny, 1824, *Sinezona cingulata* Costa, 1861) extend as far north as the Iberian Penninsula but are unknown from the British Isles, Scandinavia and north thereof, whereas anatomids (represented by *Anatoma crispata*), are well known from the British Isles, Scandinvia, Faroe Islands, and Greenland. In the Northwestern Atlantic, the northernmost scissurellids (*Sinezona confusa* Rolán & Luque, 1994, and *Satondella tabulata* Watson, 1886) are described from Bermuda, while anatomids (*Anatoma crispata*) are known from much further north. In the eastern Pacific, scissurellids (*Sinezona rimuloides* Carpenter, 1865) is found in central California, while anatomids – *Anatoma "crispata"*, *Thieleella baxteri* (McLean, 1984), *Anatoma janetae* Geiger, 2006 – are known from as far north as the Aleutian Islands. In the Western Pacific, the northernmost scissurellid is *Scissurella staminea* (A. Adams, 1862) from southern Japan, while *Anatoma disciformis* (Golikov & Sirenko, 1980) and *Anatoma* s.l. *obtusata* (Golikov & Gublin, 1978) are known from the Russian waters.

The other apparent ecological pattern found in anatomids is the strong tropical-polar diversity gradient. For instance, in the Indo-Malayan Archipelago eight species of this family are currently known, and there are approximately 25 additional undescribed species (Geiger, unpubl. data). The polar region, in contrast, only contains four anatomid species in the Antarctic, and approximately the same number in the Arctic; the only species described in the Arctic, *Anatoma crispata* Fleming, 1828, has been applied to a great multitude of species (see Geiger & Jansen, 2004a), and its application particularly to North Pacific species is erroneous (Geiger, 2006b; unpubl. data). The intermediate areas, for instance the Mediterranean-eastern Atlantic region, contain approximately eight anatomids (Geiger, unpubl. data).

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FOREST SNAIL FAUNAS FROM S. E. QUEENSLAND AND N.E. NEW SOUTH WALES (AUSTRALIA): PATTERNS OF LOCAL AND REGIONAL RICHNESS AND DIFFERENTIATION

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ABSTRACT

While regional snail faunas of low-latitude regions tend to be richer than similar areas at high latitude, there seems to be little difference between site (e.g. < 400 m²) richness, which often ranges between 20 and 40 species across a wide latitudinal range. Given this seeming paradox, it is surprising how little investigation has been made into how land snail species are packed into the tropical landscape across multiple scales. This paper addresses this question by analyzing faunal lists from six regions spread across a 550 km extent in the temperate rain forests of eastern Australia. Considerable heterogeneity was observed both within similar habitat types within (ca. 40–60% faunal similarity) and between (ca. 10–50%) different regions. The Charopidae constituted the largest fraction of the fauna (up to 70% of species), and were thus responsible for most of the variation in composition. While the number of species per region (ca. 30–50) was broadly comparable to Europe and North America, the rate of faunal turnover with distance was found to be 2–30 times greater. While geographic turnover in European and North American faunas is largely driven by large species, in eastern Australia turnover was largely driven by small, litter-dwelling species that tend to be unique to each region. The comparative richness of the eastern Australian fauna is thus largely related to evolutionary processes that have caused divergence at regional scales, rather than increased niche-packing or syntopic diversity at site scales.

Keywords: terrestrial gastropod, biodiversity, distance decay, evolution, Australia, North America, Europe.

INTRODUCTION

Recent work in many parts of the world has revealed a great variety of regional patterns in land mollusc species richness and differentiation (Barker, 2005; Cameron, 2004; Cameron et al., 2000; Nekola, 2003, 2005; Pokryszko & Cameron, 2005; Seddon et al., 2005; Stanisic, 1994; Stanisic & Ponder, 2004; Tattersfield, 1998.). These studies have tended to show that distance decay in similarity (Nekola & White, 1999) in regional faunas is low at high latitudes (Pokrysko & Cameron, 2005; Nekola, 2005), presumably due to climate fluctuations resulting in widespread extinctions of forest faunas followed by recolonization from relatively few refugia. At lower latitudes, where such widespread extinctions did not occur, patterns are more complex and reflect the interaction of local environmental gradients and history with differences in dispersal abilities and *in situ* speciation rates (Solem, 1984). These processes may allow some groups to display rapid distance decay rates while others demonstrate little. Thus, in semi-arid northwestern Australia, large and relatively stenotypic camaenids generally show dramatic patterns of allopatric replacement, while non-camaenids are ubiquitous (Solem, 1988; Cameron, 1992). In mesic subtropical New Zealand, compositional differences tend to reflect turnover among small, leaf litter dwelling charopid and punctid snails (Barker, 2005) but without clear patterns of allopatry in congenerics.

Local-scale community patterns are less well known, especially in tropical and subtropical environments where densities are often low,

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leading to acute sampling problems (Cameron & Pokryszko, 2005). Contrary to Solem's (1984) assertion, we now know that some tropical rainforest faunas are the richest known at 1 km^2 scales (Emberton, 1995; de Winter & Gittenberger, 1998; Schilthuizen & Rutjes, 2001). At even smaller scales (i.e. 0.1–1 ha), patterns are even more obscured by sampling problems. As many tropical and subtropical forests are oligotrophic and support only very low densities of snails, it is often impossible in such circumstances to distinguish between genuine local heterogeneity and sampling effects. However, such heterogeneity could be significant both for speciation events and for the co-existence of closely related species (Cameron et al., 2003).

The forests of eastern Australia have been extensively surveyed (Stanisic, 1994, 1997; Stanisic & Ponder, 2004). They constitute a complex mosaic of forest types, determined by substrate, temperature and rainfall, with a history of fluctuation in extent and connectedness. Paralleling patterns seen in E. Africa (Tattersfield, 1998; Seddon et al., 2005), individual sites may harbour up to 40 species, with considerable faunistic turnover occurring over relatively short distances. In this study, we attempt to relate local and regional variation in species richness and compositional turnover to broader regional patterns using both new surveys that attempt to overcome problems of sampling efficiency (Cameron & Pokryszko, 2005), and by reanalysis of some of Stanisic's (1997) earlier results.

STUDY AREAS AND HABITATS

Four major study areas were analyzed with multiple samples, while two outlying sites were represented by single samples (Fig. 1). The Macleay Valley in northeastern New South Wales was the subject of a major survey by Stanisic (1997), covering a geographic extent of approximately 10,000 km^2 (maximum linear distance of 70 km) with an altitudinal range extending from sea level to approximately 1,100 m. As described and referenced by Stanisic (1997), this area contains a wide variety of forest types, including:

(a) Sclerophyll Forests (SF) dominated by *Eucalyptus* species, have been historically exposed to frequent fires. While such forests can be further divided into Wet and Dry types, for these analyses, all have been lumped; both hold very poor faunas (Stanisic, 1997, and see below).

(b) Temperate Rain Forests (TRF) generally occur at high altitudes and have higher yearly precipitation levels. While we have lumped Warm and Cool subcategories together, most sites would fall in the Warm subcategory.

(c) Dry Rain Forests (DRF) occur in isolated patches at lower altitudes, and typically contain *Araucaria* species in the canopy.

(d) Coastal Rain Forests (CRF) supporting a high density of vine or liana taxa.

(e) Limestone outcrops (LO) which frequently maintain either TRF or DRF vegetation in a SF or disturbed land matrix. These sites are usually small and isolated, and provide rocky shelter and high calcium levels needed by a number of species (Stanisic, 1997). The vegetation is protected from fire by rock debris, and may be described as Vine Thicket, in which big trees are absent, but the vegetation is not fire-tolerant.

In 2004, new samples were made in the Macleay Valley in TRF, DRF, and LO. Most of these sites represent repeat sampling of sites used in Stanisic (1997), and are distinguished from these prior samples by being referred to as "Kempsey".

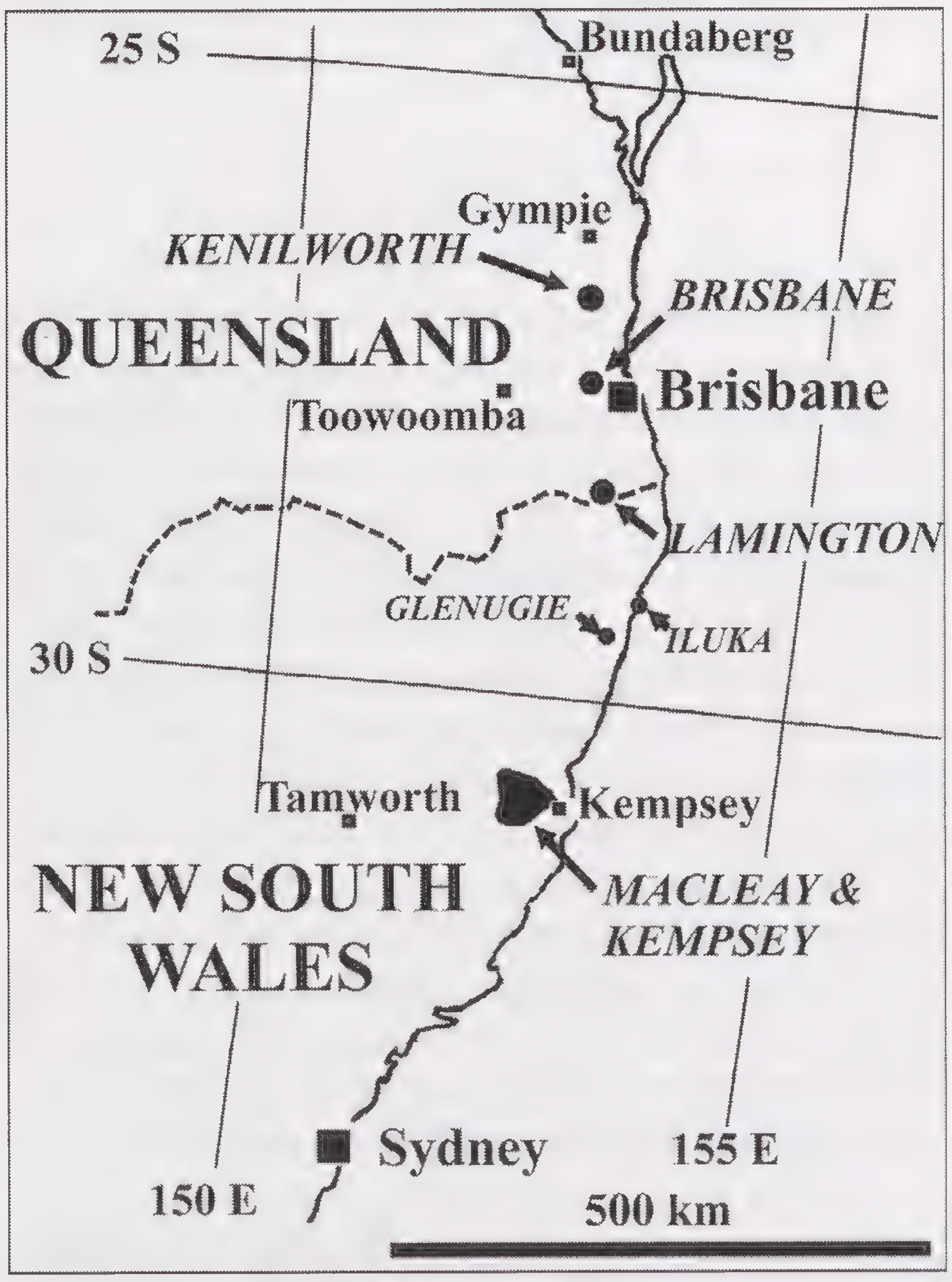

FIG. 1. Locations of study areas (italic upper case) in SE Queensland and NE New South Wales.

The three other major sampling areas were in southeastern Queensland. Samples near Kenilworth were made on the fringes of Conondale National Park in the valley and low hills surrounding the Mary River, with an outlier at Mapleton. Maximum linear extent was c. 10 km (Mapleton, ca. 30 km) with an altitudinal range from 120–250 m. All but one site were in DRF, the exception (site 3) being in Riverine Rain Forest (RRF) with large strangler figs, *Ficus watkinsiana*. Samples from Brisbane Forest, all collected within 10 km of each other, come from patches of Rain Forest near hill summits. All but one site (all above 650 m) were classified as Wet Rain Forest (WRF), the exception (site 5) being in DRF at c. 450 m. Samples from Lamington National Park, in the Border Ranges, fall into two categories: two at approximately 800 m in WRF, and two below 500 m in DRF. All sites are within 5 km of each other. Maximum linear extent (Kenilworth to Kempsey) was approximately 550 km. Two additional samples were made between the most southerly of the Queensland areas (Lamington) and the Macleay valley/Kempsey. At Glenugie, the sample was made in DRF and Vine Thicket on basaltic talus slopes. At Iluka, the sample came from a patch of CRF.

METHODS AND MATERIALS

Details of sampling methods used in the Macleay Valley survey are given in Stanisic (1997). Collections involved both searching by eye and the collection and subsequent searching of litter, but sampling intensity was not uniform between sites. From the sites sampled, we have chosen for further analysis 69 in which the habitat is unambiguously in one of the five categories described above, and in which at least two species were recorded. Sites from "woodland" or in other disturbed or mixed habitats are excluded. These lack many of the species found in less disturbed vegetation (Stanisic, 1997). The set of samples used here nevertheless includes all the native species found in the original survey.

In 2004, sites in the Macleay Valley were selected to be representative of the major habitats described above, excluding SF and CRF. In the other regions examined, sampling concentrated on the dominant rainforest categories. The area searched varied between c. 400 m^2 and 1 ha. Searches involved between two and four person-hours. About 10 l. of litter was collected and sieved at each site; coarse material was examined *in situ,* and the remainder was bagged and examined in the laboratory. All identifiable shells were extracted, identified and counted. Some large and easily identified species were counted in the field and left *in situ*.

All identifications were carried out by J. Stanisic or D. Potter. Where morphospecies are recognised, but not yet formally described, they are given a reference number connected to examined and documented lots in the Queensland Museum. Nomenclature of described species follows Stanisic (1997). Lots constituting new locality records are retained by the Queensland Museum with other material deposited in the Museum of Natural History, Wrocław, and in the National Museum of Wales, Cardiff.

Similarities between pairwise combinations of faunas have been analysed using the Nei index, in which the number of species in common is divided by the geometric mean of richness between both faunas. This statistic has a linear relationship to single step changes in similarity (Pokryszko & Cameron, 2005) unlike the familiar Jaccard index (Nekola & White 1999), which is concave. For the Macleay Valley survey, we have also considered similarity as the proportion of common species to the least rich member of the pair, following exclusion of all species recorded only from single sites, as such unique species only add to dissimilarity. In all analyses, we attempt to take sampling error (Cameron & Pokryszko, 2005) into account, chiefly by a conservative approach to the analysis of differences.

We have used Whittaker's Index ($I_w = S/\alpha$, where S = number of species recorded for the region and/or habitat within it, and α = mean number of species per site within that category), and its variant (I_{max} = S divided by the number of species in the richest site recorded), as indicators of faunal differentiation between sites in the same region (Cameron & Pokryszko, 2004). The latter index eliminates the effect of site faunas that are merely impoverished versions of others, rather than containing distinctive species not found elsewhere.

TABLE 1. Basic data for the 69 sites used from the Macleay Valley survey (Stanisic, 1997). Singletons, species found in only one site overall; Single/habitat, species found only in one site in that habitat. 50%+/habitat, species found in half or more of sites in that habitat.

	SF	TRF	LO	DRF	CRF	Total
Number of Sites	17	22	9	15	6	69
Number of Species	28	55	71	62	27	112
Mean no. of species/site	3.64	7.95	27.9	12.73	9.5	
Standard Deviation	1.5	4.11	6.45	5.14	4.18	
Range of no.s of species/site	2–6	3–17	21–40	5–22	4–14	
I_w	7.69	6.92	2.54	4.87	2.84	
I_{max}	4.67	3.23	1.78	2.82	1.93	
Number of Unique species	3	16	18	11	1	49
Number of singletons	2	10	9	7	1	29
Single/habitat	15	25	21	16	9	
50%+/habitat	0	3	21	3	9	

RESULTS

Macleay Valley Survey

Appendix 1 lists, by habitat, the species-level taxa found among the 69 sites considered. These include all species reported in Stanisic (1997). Details of site locations and faunas are available at the Queensland Museum, or in electronic form from the authors. Table 1 shows the basic data for these sites by habitat. A total of 112 species, including five introduced species (* in Appendix), were found. None of the introduced species were abundant or widespread.

As shown by Stanisic (1997), SF sites have the poorest faunas, and LO the richest. The values of I_w and I_{max} show that recorded SF and TRF forest faunas are exceptionally heterogeneous, while those of LO and CRF are much less so. The high level of heterogeneity among sites is also shown by the high proportion (29 species, 26%) of species recorded from only one site, and by the low proportions of species that occur in at least 50% of the sites in any one habitat. Strikingly, no species occur in more than half of the SF sites, and only in LO and CRF sites are there more than 10% of species in this category. Conversely, 45% or more of species occur only once within SF or TRF sites, while 33% or fewer of species occurred only once in LO, DRF and CRF sites. Among the habitat faunas, only one species is unique to CRF, three to SF, while LO harbours 18. Only 10 species were observed within all five habitats. Of the 41 species missing from LO sites, most are so infrequent (20 have only single reported occurrences) that their absence from limestone sites may be attributed to sampling error, given the small number (9) of observed sites.

Table 2 shows the matrix of similarities between habitats. The Nei index demonstrates that the strongest connection is between LO and DRF, whereas the maximum similarity index identifies that CRF sites are most related to LO and DRF faunas. Both indices strongly indicate that SF faunas are differentiated from CRF and LO.

For the overall fauna, the Charopidae are much more numerous (55 species, 49%) than

TABLE 2. Similarity matrices for between-habitat comparisons in the Macleay Valley using (A) the Nei index, and (B) taking the number of species in common (excluding those recorded once only) as a proportion of those in the poorest habitat (ex unique). Values expressed as percentages.

A: Nei	TRF	LO	DRF	CRF
SF	48	38	48	47
TRF		50	53	44
LO			65	53
DRF				56

B: Ex unique	TRF	LO	DRF	CRF
SF	73	65	77	50
TRF		69	69	65
LO			78	88
DRF				88

TABLE 3. Numbers and proportions of species in Charopidae in Macleay Valley sites, by habitat, by position in the quartiles of overall frequency, and by habitat range (number of the five habitats at left in which the species were recorded). Quartiles differ slightly in size because of ties in frequency. Note that the bottom quartile (4) consists of species recorded in only one site overall.

Charopidae	No.	%
SF	8	29
TRF	27	49
LO	28	39
DRF	24	39
Total	55	49

Quartile	Total species	Charopidae	%
1	30	10	33
2	29	12	41
3	24	13	54
4	29	21	72
Habitats	Total species	Charopidae	%
1	49	33	67
2	25	10	40
3–5	38	12	32

any other family (Table 3). SF has the smallest proportion of charopids in its fauna (8, 29%) while TRF has the largest (27, 49%). Nearly three quarters of all single-site species are charopids, as are two thirds of all single-habitat specialists. This single family thus accounts for most of the observed differences between habitats and among sites within habitats.

2004 Surveys

Appendix 2 lists the 149 species level taxa found by survey area. Six of the species recorded are introduced (* in Appendix). Of these, only *Lamellaxis clavulinus* was recorded in large numbers, and then only in one site. Full details of sites, and of the species composition of samples from each are deposited at the Queensland Museum and are available electronically. Table 4 shows the basic data for each survey area. Results for Kempsey are split by habitat, as the range of habitats sampled was much greater than elsewhere, and the LO sites are very different from all others. Of the categories with more than one sample, only in Kempsey LO do all sites meet the minimum requirements of at least 10 times as many individuals as species and at least 200 individuals, and in Lamington and Brisbane virtually no site meets these criteria (Cameron & Pokryszko, 2005). Logarithmic rank/abundance curves tend to be concave in Brisbane and in some Kempsey habitats, with a tail of very rare species. They are more convex in Lamington and Kenilworth, where the pattern is very similar to that recorded for non-limestone Carpathian forests in central Europe (Fig. 2). Within the Kenilworth set, there is also

TABLE 4. Basic data for each area in the 2004 survey. Ind/site, mean number of individual shells collected or recorded per site. For habitat abbreviations, see text.

	Kenilworth DRF/RRF	Brisbane WRF/DRF	Lamington WRF/DRF	Kempsey LO	Kemp DRF	Kemp TRF	Glenugie DRF	Iluka CRF
Sites	10	6	4	3	3	1	1	1
Species	49	34	41	51	36	11	18	11
Mean/site	24.1	13.5	22.3	28.0	17.0	11	18	11
SD	4.4	3.2	7.9	1.7	6.2	N/a	N/a	N/a
Range	17–32	9–17	13–32	27–30	10–22	N/a	N/a	N/a
I_w	2.03	2.52	1.84	1.82	2.12	N/a	N/a	N/a
I_{max}	1.53	2.00	1.28	1.70	1.64	N/a	N/a	N/a
Ind/site	295	135	122	3033	136	35	433	248
SD	162	46	51	2911	66	N/a	N/a	N/a
Range	109–558	92–218	72–185	369–6141	60–185	N/a	N/a	N/a

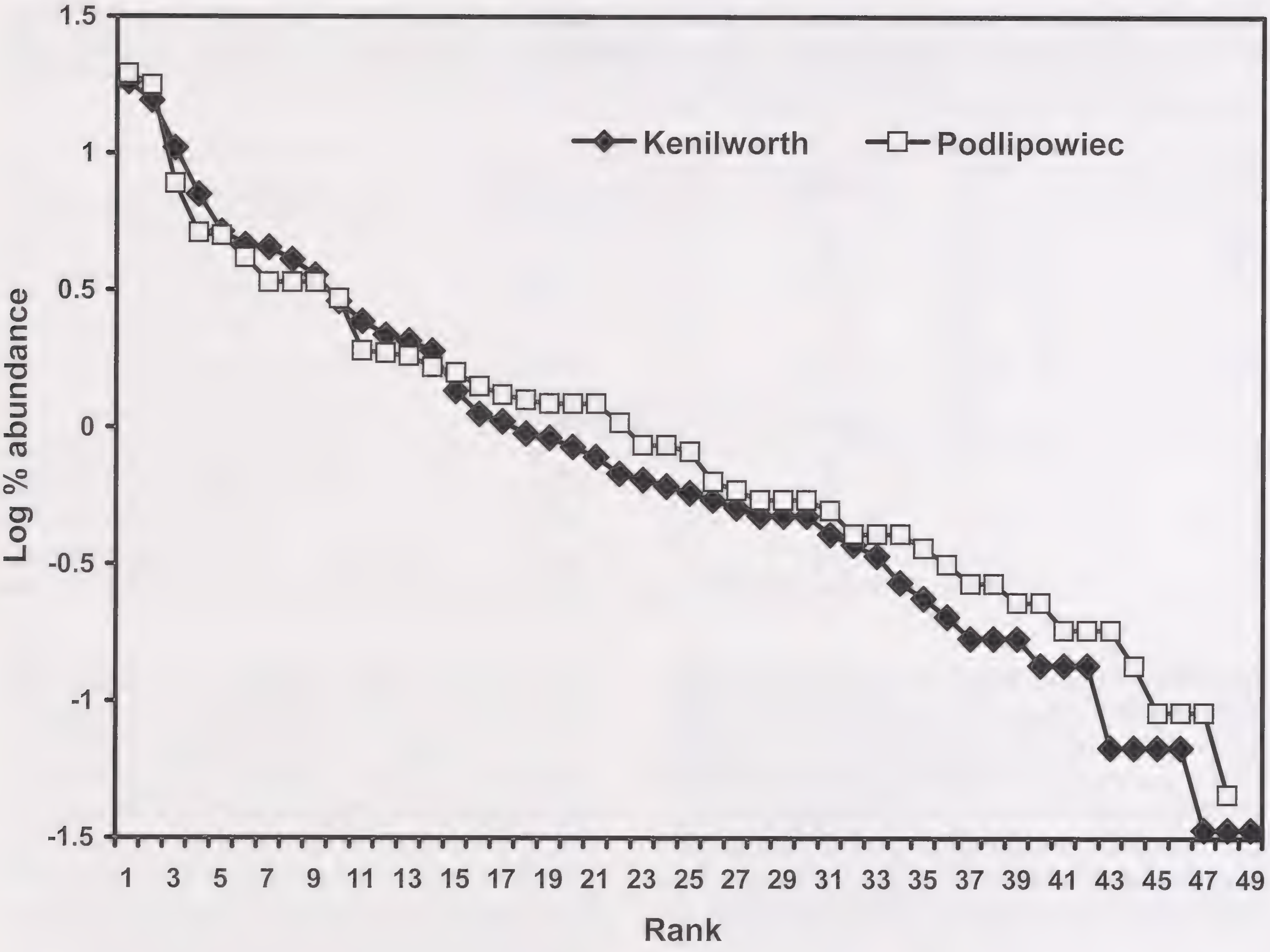

FIG. 2. Logarithmic plot of relative species abundances (%) for the snail faunas in samples from Kenilworth, Queensland, and from Carpathian beech forests at Podlipowiec, S. Poland (Pokryszko and Cameron, unpublished).

no significant correlation between numbers of species and of individuals (R = 0.22, P > 0.2, n = 10). Thus, with that exception, we may suspect that recorded site faunas are significantly incomplete. Nevertheless, the range of site richness, and the values of I_w and I_{max} are generally much lower than those seen within habitats in the Macleay survey.

These indices are larger in Brisbane than elsewhere. In this area, five of the six sites are characterised as WRF, all above 650 m asl. The other site, DRF at c. 450 m, harbours six unique species, and is one of the two richest sites across the entire survey. Without this site the WRF sites of Brisbane have a mean of 12.8 species/site and I_w and I_{max} values of 2.19 and 1.64 respectively, more in line with values for other areas. A similar pattern is evident at Lamington, where the two lower elevation DRF sites (with *Araucaria*) were richer in species and individuals than the two higher elevation WRF sites. Because of limited number of samples, however, not enough statistical power exists to test hypotheses regarding this pattern. At Kenilworth, one site (Kenilworth 3) comes from Riverine Rain Forest, but shows no peculiarity in richness or native species composition from the remaining DRF sites, though it contained large numbers of the introduced *Lamellaxis clavulinus* (Subulinidae).

Charopidae are the most numerous family in terms of species. As in the Macleay survey, they generally contribute to differences (rather than similarity) between areas, except in the single-site cases of Glenugie and Iluka. Omitting these two, the overwhelming majority of species are found in only one region, and only three (2.1%) are found in all four.

Table 5 shows Nei similarity index values within and between regional habitat and total faunas. Fig. 3 shows the dendrogram of similarities between sites generated by UPGMA clustering. The Kenilworth faunas are the most uniform, with an average inter-sample similarity of 64% in the region. Strong distance

TABLE 5. Values of the Nei index of similarity (%) in the 2004 survey, A: on mean site-by-site comparisons within and among areas and habitats; B: among the total faunas of each area.

A: Nei, mean	Kenilworth	Brisbane	Lamington	Kempsey DRF	Kempsey LO	Kempsey TRF	Glenugie	Iluka
Kenilworth	64	32	20	5	6	6	13	8
Brisbane		55	44	12	9	21	14	6
Lamington			55	11	4	16	6	5
Kempsey DRF				40	29	30	9	4
Kempsey LO					51	20	14	17
Kempsey WTRF						n/a	14	9
Glenugie							n/a	22
Iluka								n/a

B: Nei, total	Brisbane	Lamington	Kempsey	Glenugie	Iluka
Kenilworth	52	34	18	14	29
Brisbane		51	17	21	10
Lamington			13	12	5
Kempsey				15	15
Glenugie					22

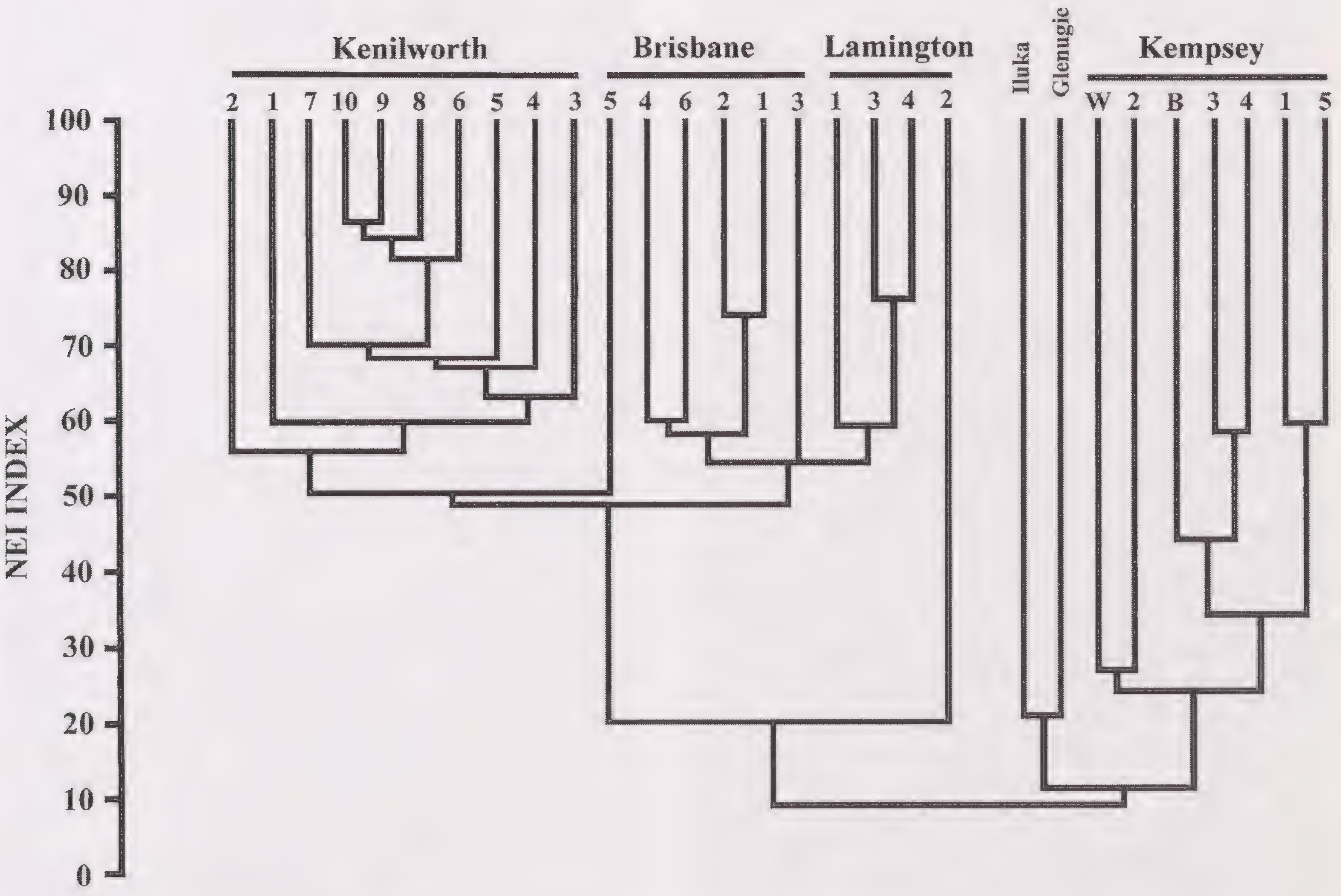

FIG. 3. Dendrogram of Nei index similarities for 2004 study sites (UPGMA). In Kempsey, sites 1, 4 and 5 are from limestone outcrops; W (WayWay Forest) and B (Bob's Road) are based on very small samples (see text).

TABLE 6. A comparison of the numbers of species found in the original Macleay Valley survey and in 2004 for six sites (see text). Kempsey 4, 1 and 5 are limestone sites, 6 and 7 are DRF, 2 is TRF.

Species	Natural Arch Kempsey 4	Yessabah Kempsey 1	Mt Pleasant Kempsey 5	Blowhole Kempsey 7	Way Way Kempsey 6	Bob's Rd Kempsey 2
Macleay (JS)	35	40	32	14	22	8
2004	30	27	27	19	10	11
Present both	25	25	21	12	9	6
Aggregate	39	42	36	21	23	13
Nei index (%)	78	76	74	74	61	64

decay is apparent between regional faunas, with only 6% similarity being noted over the 550 km extent from Kenilworth to Kempsey. All Queensland areas have low affinities to New South Wales areas (4–16%), while similarity between New South Wales sites also are surprisingly low (4–22%). While the dendrogram also generally demonstrates this same pattern, it also shows that the Brisbane DRF habitat resembles Kenilworth DRF sites. Thus, all six species unique in the Brisbane area to this single DRF site are held in common with one or more Kenilworth DRF sites.

The comparison of total study area faunas may provide more robust results by summing across multiple samples, increasing the number of encountered microenvironments and individuals (Nekola & White 1999). Similarity patterns for total faunas at this scale demonstrate essentially an identical pattern, with similarity falling to 14% between Kenilworth and Glenugie. Similarity of Kenilworth and Kempsey non-limestone sites fell to 6.3%. While Queensland sites maintained somewhat higher similarities (34–52%), inter-area similarity south from Lamington generally ranged from 12 to 15%. The least similarity was noted between the faunas of Lamington and Iluka (5%). Thus, the New South Wales sites are equally dissimilar to each other as they are to the Queensland areas. This can be explored in more detail (Table 4). With the exception of Brisbane, roughly midway between Lamington and Kenilworth, more than 40% of recorded species were unique to each region. The highest proportion of unique species (78%) was observed at Kempsey. While this may represent the presence of base-rich limestone habitats, geographic factors also appear important as the frequency of unique species within the Kempsey non-limestone fauna remains significantly higher (68%) than that observed from all other sites.

Macleay- Kempsey Comparisons

Six of the seven Kempsey sites sampled in 2004 had been visited in the earlier survey. A comparison of results is thus possible (Table 6). Although areas searched do not exactly correspond, the differences between occasions show that even when sample sizes are large (Kempsey 1 produced more than 6,000 shells), not all the fauna is necessarily detected. Nevertheless, indices of similarity for repeat samples at the same site are higher than those generally recorded between sites in the same habitat. It is clear that one sample, Kempsey 6, Way Way Forest, was seriously deficient in 2004, with only 60 individuals and 10 species retrieved. This is reflected in its distant relationship to all other sites in Figure 2. Using the combined data for each Kempsey/ Macleay site increases levels of between site similarities within each habitat (data not shown). The combined total numbers of species in each of these sites match the maxima for those habitats recorded in the earlier survey quite closely. Overall, the 2004 survey recorded only 67 species in Kempsey, or 60% of those recorded in the larger Macleay Valley survey. As the two habitats not examined in 2004, SF and CFR, hold few unique species, thus lower number mainly reflects the smaller number of samples made and the smaller proportion of the original survey area sampled. While the earlier Macleay survey clearly gives a more comprehensive view of the fauna of each habitat, it does not identify much different rates of turnover with other faunas. For example, similarity between DRF in the 1997 Macleay survey and Kenilworth provided a Nei Index of 16.7% versus 18% for the 2004 Kempsey data. Deficiencies in sampling are thus not seriously distorting similarity estimates between faunas.

DISCUSSION

Reliability and Sampling Error

It is clear that in both the 1997 Macleay Valley and 2004 Kempsey surveys, site faunas were incompletely inventoried (Cameron & Pokryszko, 2005), largely due to the low densities of individuals on non-limestone sites. As shown by the very high values of $I_{w,}$ I_{max}, and the large proportion of singleton records, this problem was most acute in the 1997 survey, with some of this error reflecting variation in sampling intensity between sites. In the 2004 standardised surveys, this problem remained, but was less severe. Only Kenilworth and Kempsey LO sites were adequately sampled based on total observed richness and abundance (Cameron & Pokryszko, 2005). However, comparisons with the 1997 survey in Macleay/Kempsey suggest that even samples of approximately > 6,000 shells may not completely inventory all taxa, or that very small shifts in location may add more species to an inventory, even within the same habitat. The logistics of quadrupling sampling effort at each site are likely impossible within reasonable time frames. In spite of these limitations, 2004 site data compare favourably with most other tropical studies (Emberton, 1995; de Winter & Gittenberger, 1999; Schilthuizen & Rutjes, 2001), and are similar to those recorded by Tattersfield (1996) in Kakamega Forest, Kenya, where supporting evidence suggests that the aggregate fauna has been adequately inventoried (Cameron & Pokryszko, 2005; Tattersfield et al., 2006). The possible effects of such sampling error on diversity estimates are considered below where relevant. Compared to the traditional Jaccard index, the Nei index of similarity gives some compensation for differing richness in compared sites. Estimates (data not shown) based on the proportion of species held in common by the poorer of two sites shows the same pattern as that revealed by the Nei index. Since we are more concerned with faunal differences, than with richness *per se,* we have refrained from using rarefaction or species accumulation curves, as these give no indication of which taxa might have been overlooked.

Habitat Effects and the Range of Local Richness

Stanisic (1997) demonstrated in the Macleay Valley that SF was the least rich at site level, while LO was the richest. Our 2004 surveys replicate that finding, and allow estimation of maximum richness levels for each habitat studied. At the site level, LO site maxima exceed 40 species. That the total fauna of LO sites is considerably higher in the original 1997 (71 species) and in the more limited 2004 survey (51 species) reflects both environmental differences between LO habitats and the presence of single outcrop endemics (Stanisic, 1997). Using data from both 1997 and 2004 where possible, DRF off limestone have maxima of 22–23 taxa/site, TRF and CRF have maxima from 15–17, while SF has a maximum of 6. SF and CRF, both base-poor habitats, appear to simply harbour reduced versions of the faunas found in base-rich habitats, as they support very few unique species, most of which are known from only single records. While more intensive work might raise these maximum richness figures slightly, it seems likely that greater between-site heterogeneity in base-poor habitats reflects a genuine patchiness in species distributions at scales larger than sample sites. We cannot tell at present whether this heterogeneity reflects small-scale geographical differentiation within the valley.

In the Queensland series, evidence suggests that WRF supports less rich faunas as compared to DRF, especially in Brisbane and Lamington. For WRF, richness appears to range up to 25 per site, and to 35 over larger areas of a few km^2. However, DRF can support more than 30 species in single sites both in Lamington and Kenilworth, while they appear to range up to 40–50 over larger areas.

These local richness levels are similar to those found in forest faunas from tropical East Africa (Tattersfield et al., 2006; Seddon et al., 2005*)*, but the maxima are rather less than those found on the North Island, New Zealand (up to 64 in a site, perhaps 75+ in a few ha, Barker, 2005). While rainforests in Madagascar, Cameroon and Borneo are richer at larger scales (60–110 species per 1 km^2; Emberton, 1995; de Winter & Gittenberger, 1998; Schilthuizen & Rutjes, 2001), at the scale of 400 m^2 plots accurate comparisons are not possible due to sampling errors (Cameron, 2004; Cameron & Pokryszko, 2005). Surprisingly, the range in site richness values is remarkably similar (2–45 species/site) to that recorded in European (Pokryszko & Cameron, 2005) and eastern North American (Nekola & Smith, 1999; Nekola, 2005) temperate forests. Thus, maximum site richness of forest faunas shows little global latitudinal variation, despite great and deep taxonomic differences. This observation also holds more generally within eastern Australia, where sites holding c. 35–40 species can be found at widely separated latitudes (Stanisic, 1994; Stanisic & Ponder, 2004).

The pattern in all four of our study areas seems to conform to Waldén's (1981) conclusion that within a given forest area poor sites have merely reduced versions of richer ones. In areas where a greater altitudinal range is sampled, this not the case, with some species being restricted to higher or lower altitudes (Stanisic 1982, Tattersfield et al., 2006). Hence, the inclusion of poorer forest types in some but not all of our regional comparisons does not invalidate the estimation of faunal similarities and turnover.

Faunal Turnover and Taxonomic Composition

There is a considerable amount of faunal turnover over the approximately 550 km distance between our most distant sites. Table 7 demonstrates this by comparison with non-limestone regions along the USA-Canada border (Nekola, 2005, and unpublished), the Sudetes-Carpathian chain in southern Poland (Pokryszko & Cameron, 2005, and unpublished), and the southern Rocky Mountain cordillera in the southwestern USA (Nekola, unpublished). Except for Brisbane, rates of uniqueness in the local eastern Australian faunas were greater than 40%. Outside of eastern Australia, only one other region, the Chiricahua Mountains (Arizona), exceeded this level. All remaining sites demonstrated uniqueness levels < 20%. Given the continuous nature of compositional turnover (Whittaker, 1975; Nekola & White, 1999), the arbitrary transect endpoints should harbour the most unique species, as was observed in eastern Australia, Poland, and the southern Rockies. The higher rates of unique species in the centre of the northeastern USA transect is due to the incursion of southern species into buffered regional climates adjacent to, or immediately downwind of the Great Lakes.

The Nei endpoint similarity was also much lower for the eastern Australian transect (16.3) than for either of the mountain transects (southern Rockies = 41.7, Carpathians = 46.7) or for the northeastern USA (81.3). Assuming that similarity falls in a constant exponential fashion with interregional distance (Nekola & White, 1999), these values indicate that only 190 km is required for faunistic similarity to fall by 50% in eastern Australia, while it will

TABLE 7. Comparative data on turnover and faunal differences for four sets of sites on non-calcareous soils in E. Australia, NE USA, Poland, and SW USA (see text). Kempsey data exclude sites on limestone. The NE USA and Polish sequences run from west to east, the Australian and SE USA from north to south. The Nei ends column gives the value of the Nei index between the two faunas at the ends of each sequence, which are about 500 km distant except in the NE USA where they are 1,800 km apart.

E. Australia	*Kenilworth*	*Brisbane*	*Lamington*	*Kempsey*	Nei, ends
No. of species	49	34	41	38	16.3
Unique	20	4	18	26	
% Unique	40.8	11.8	43.9	68.4	
NE USA	*Duluth, MN*	*Copper Harbor, MI*	*Northern Vermont*	*Northern Maine*	Nei, ends
No. of species	23	33	29	19	81.3
Unique	0	6	2	0	
% Unique	0	18.2	6.9	0	
Poland	*Mysliborski*	*Mlynowiec*	*Podlipowiec*	*Ustrzyki*	Nei, ends
No. of species	37	37	48	46	46.7
Unique	7	4	5	9	
% Unique	18.9	10.8	10.4	19.7	
SW USA	*Chiricahua Mts.*	*Sierra Blanca Mts.*	*Southern Sangre de Christo Mts.*	*San Juan Mts.*	Nei, ends
No. of species	25	18	20	23	41.7
Unique	12	2	3	4	
% Unique	48.0	11.1	15.0	17.4	

take 400 km in the southern Rockies, 450 km in the Polish Carpathians, and 6,000 km in the northeastern USA. The eastern Australian fauna is thus experiencing geographic turnover at 2.1–31.6 times the rate experienced by these other faunas, helping explain how the outlying Glenugie and Iluka sites demonstrate such high degrees of uniqueness: in eastern Australia the similarity of regions separated by only 100 km would be expected to fall by 30%.

The Australian situation is similar, though not as extreme, as that reported by Tattersfield (1998) and Tattersfield et al. (1998) for Indian Ocean coastal forests and Eastern Arc Mountains in East Africa. In eastern Australia, single-site endemics are unusual except for isolated limestone outcrops (Stanisic & Ponder, 2004). Most species for which we have reliable information have linear ranges between 50 and 200 km (Stanisic, 1990), and these are not always coherent. In this respect, these faunas resemble those of North Island, New Zealand (Barker, 2005), and are very different from those in the semi-arid northwest of Australia, where large camaenid snails often have very restricted ranges (of the order of 1–10 km), and show strict patterns of within-genus allopatric replacement (Solem, 1988; Cameron et al., 2005). Although camaenids and other large-shelled families (Rhytididae and Helicarionidae) do contribute to faunal differentiation in E. Australian forests, it is clear that the much smaller, litter-dwelling Charopidae contribute most, as they do in New Zealand. In Europe and North America small litter dwelling species tend to unify the composition between regions as they have large geographical ranges (Pokryszko & Cameron, 2005). However, in these regions it is generally the large helicoids and clausiliids (Europe) and large polgyrids, endodontids, and helminthoglyptids (North America) that differentiate faunas.

In terms of taxonomic composition, we note that introduced species are only a small element of these faunas, and the few species involved are not usually abundant. While many of the faunas we have examined may be affected by human activities, they have so far retained their integrity as indigenous assemblages.

Pattern and Process

Although interpretation is complicated by sampling errors in many studies (including this one) certain patterns can be discerned and reasonably attributed to known environmental fluctuations that have influenced the degree of habitat connectedness. In both N.W. Australia and eastern Africa, climatic fluctuations within the Pliocene/Pleistocene have periodically isolated favourable forest habitats, within which stenotypic species have been confined and differentiated (Cameron, 1992; Tattersfield, 1998). Similar proliferation of allopatric sister species occurs elsewhere, for example in the Aegean region of Europe (Douris et al., 1998; Parmakelis et al., 2005). In eastern Australia, periodic isolation of mesic forest habitats during the Pleistocene (Kershaw, 1981, 1994; Lloyd & Kershaw, 1997; Harle, 1997) has constantly altered the extent and connectedness of forest types. While allopatric speciation can be pronounced on isolated limestone outcrops (Stanisic & Ponder, 2004), as in New Zealand (Barker, 2005), few examples of strict allopatry or parapatry between congeners are apparent, especially among the speciose charopids. The presence of co-existing congeners in eastern Australia thus suggests that speciation occurred within small regions, as has been suggested in eastern Africa (Tattersfield, 1998). The presence of altitudinal zonation in the eastern Australian fauna (i.e., Bellenden Ker, Stanisic, 1982) also suggests that some speciation has been driven, as in Africa (Seddon et al., 2005), by topographic complexity.

When considering the very high local diversity of some New Zealand sites, also dominated by small charopids and punctids, Solem (1983, 1984) suggested that this diversity had built up slowly in a very stable environment. By implication, this process involved isolation by distance (which can be modest, given their low mobility), differentiation, and back colonisation into ancestral territory that was still occupied. This process implies that competitive exclusion is relatively unimportant, and that even nearby populations may be sufficiently isolated to allow for differentiation. With the evidence of competitive exclusion in land molluscs being meagre, it is worth noting that a metapopulation structure involving small, temporary and shifting populations allows local co-existence of ecological equivalents (Hubbell, 2001). These dynamics may be important for some tropical and subtropical faunas that consist of low-density populations that are patchily distributed around temporarily favourable microhabitats, such as coarse woody debris (Cameron et al., 2003). As with estimates of local richness, further investigation of such mechanisms will encounter formidable sampling problems. While this current study certainly does not overcome these problems, the data it generated do not contradict the expected patterns generated by these processes.

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APPENDIX 1

Species recorded by Stanisic (1997) in the Macleay Valley, ordered by overall frequency of occurrence, and showing the number of sites occupied in each habitat. For abbreviations, see text. Asterisked species are introduced.

		SF	TRF	LO	DRF	CRF	TOTAL
Species	Sites	17	22	9	15	6	69
Gyrocochlea planorbis		7	13	8	6	5	39
Saladelos macquariensis		5	5	7	10	3	30
Austrochloritis nambucca		6	11	3	3	6	29
Rhytididae MV3		4	9	7	4	2	26
Rhytididae MV7		1	10	3	6	3	23
Thersites novaehollandiae		2	7	4	7	1	21
Coenocharopa multiradiata		0	4	7	9	1	21
Pleuropoma jana		0	1	9	7	3	20
Helicarionidae MV5		5	1	8	3	3	20
Nitor medioximus		0	3	3	10	3	19
Punctidae MV1		0	1	8	5	1	15
Rhytididae MV1		0	2	5	5	3	15
Hedleyella falconeri		0	13	1	1	0	15
Iotula microcosmos		0	2	7	4	1	14
Charopidae MV35		2	3	0	7	2	14
Parmavitrina planilabris		1	8	1	4	0	14

(continues)

(continued)

		SF	TRF	LO	DRF	CRF	TOTAL
Species	Sites	17	22	9	15	6	69
Helicarionidae MV1		1	5	2	6	0	14
Allocharopa belli		1	10	0	2	0	13
Tornatellinops jacksonensis		0	1	9	3	0	13
Charopidae MV33		2	4	3	2	2	13
Pupisoma circumlitum		0	0	8	4	0	12
Pupillid MV1		0	1	7	4	0	12
Hedleyoconcha delta		0	3	3	3	3	12
**Discocharopa aperta*		0	0	8	2	1	11
Charopidae MV30		0	0	6	4	0	10
Gastrocopta bifurcata		0	0	7	3	0	10
Charopidae MV22		2	1	4	1	2	10
Camaenidae MV2		3	0	5	0	2	10
Tornatellinops pressus		0	0	5	2	2	9
Cralopa stroudensis		1	2	2	4	0	9
Charopidae MV6		0	0	4	2	2	8
Georissa laseroni		0	0	8	0	0	8
Charopidae MV4		0	0	6	2	0	8
Charopidae MV23		0	0	3	5	0	8
Helicarionidae MV4		1	7	0	0	0	8
Gastrocopta queenslandica		0	0	5	2	0	7
Rhophodon kempseyensis		0	0	7	0	0	7
Camaenidae MV9		4	0	0	2	1	7
Glyptopupoides egregia		0	0	4	2	0	6
Pygmipanda atomata		1	4	1	0	0	6
Coneuplecta calculosa		0	1	2	3	0	6
Camaenidae MV11		1	0	3	2	0	6
Elasmias wakefieldiae		0	0	3	0	2	5
Triboniophora graeffei		1	0	1	1	2	5
Rhytididae MV2		0	3	2	0	0	5
Charopidae MV25		0	1	4	0	0	5
Charopidae MV38		1	3	0	1	0	5
Helicarionidae MV2		0	0	4	1	0	5
Camaenidae MV4		3	0	0	2	0	5
Camaenidae MV7		0	1	1	3	0	5
Cystopeltidae MV1		1	1	0	2	0	4
Charopidae MV7		0	0	4	0	0	4
Charopidae MV24		0	0	1	3	0	4
Charopidae MV27		0	4	0	0	0	4
Charopidae MV31		0	0	2	1	1	4
Charopidae MV46		0	0	4	0	0	4
Posorites conscendens		0	1	2	1	0	4
Camaenidae MV8		1	0	3	0	0	4
Camaenidae MV12		0	0	0	4	0	4
Charopidae MV18		0	2	1	0	0	3
Charopidae MV2		0	0	0	3	0	3
Charopidae MV16		0	0	1	2	0	3
Charopidae MV36		1	2	0	0	0	3
Helicarionidae MV3		0	1	0	2	0	3

(continues)

(continued)

Species	Sites	SF 17	TRF 22	LO 9	DRF 15	CRF 6	TOTAL 69
Camaenidae MV5		0	3	0	0	0	3
Camaenidae MV6		0	0	1	2	0	3
Camaenidae MV10		0	1	2	0	0	3
Gastrocopta pediculus		0	0	2	0	0	2
**Paralaoma caputspinulae*		0	0	2	0	0	2
Rhytididae MV4		0	0	0	2	0	2
Ngairea dorrigoensis		0	2	0	0	0	2
Letomola contortus		0	0	2	0	0	2
Charopidae MV1		0	0	2	0	0	2
Charopidae MV9		0	1	0	1	0	2
Charopidae MV13		0	0	2	0	0	2
Charopidae MV26		0	1	1	0	0	2
Charopidae MV34		0	2	0	0	0	2
Charopidae MV40		0	0	0	2	0	2
Charopidae MV44		0	2	0	0	0	2
Liardetia scandens		0	0	1	0	1	2
Austrochloritis brevipila		0	2	0	0	0	2
Camaenidae MV3		2	0	0	0	0	2
**Bradybaena similaris*		0	0	1	1	0	2
Cylindrovertilla kingi		0	0	1	0	0	1
Pupillid MV2		0	0	1	0	0	1
**Lamellaxis clavulinus*		0	0	0	1	0	1
Succineidae MV1		0	0	1	0	0	1
Rhytididae MV6		1	0	0	0	0	1
Coenocharopa yessabahensis		0	0	1	0	0	1
Egilomen lirata		0	1	0	0	0	1
Charopidae MV3		0	1	0	0	0	1
Charopidae MV5		0	0	1	0	0	1
Charopidae MV8		0	0	1	0	0	1
Charopidae MV10		0	0	0	1	0	1
Charopidae MV11		0	0	0	1	0	1
Charopidae MV12		0	0	0	0	1	1
Charopidae MV14		0	0	1	0	0	1
Charopidae MV15		0	0	0	1	0	1
Charopidae MV19		0	1	0	0	0	1
Charopidae MV20		0	1	0	0	0	1
Charopidae MV28		0	0	1	0	0	1
Charopidae MV29		0	0	0	1	0	1
Charopidae MV32		0	1	0	0	0	1
Charopidae MV37		0	1	0	0	0	1
Charopidae MV39		0	1	0	0	0	1
Charopidae MV41		0	1	0	0	0	1
Charopidae MV42		0	1	0	0	0	1
Charopidae MV43		0	1	0	0	0	1
Charopidae MV45		0	0	1	0	0	1
Papuexul bidwilli		0	0	0	1	0	1
Camaenidae MV1		1	0	0	0	0	1
**Zonitoides arboreus*		0	0	0	1	0	1
Total Species		28	55	71	62	27	112

APPENDIX 2

Occurrence of species in each area sampled in 2004. Asterisked species are introduced.

	Kenilworth	Brisbane	Lamington	Glenugie	Iluka	Kempsey
Georissa laseroni	0	0	0	0	0	X
Pleuropoma jana	0	0	0	0	0	X
Pleuropoma draytonensis	X	X	X	0	0	0
Pupina wilcoxi	X	0	0	0	0	0
Pupina strangei	X	0	0	0	0	0
Pupinidae NN1	0	0	0	X	0	0
Pupinidae BR1	X	0	0	0	0	0
Pupinella costata	X	0	0	0	0	0
Velepalaina strangei	0	0	X	0	0	0
Tornatellinops jacksonensis	0	X	0	X	X	X
Tornatellinops pressus	0	0	0	0	0	X
Elasmias wekefieldiae	0	0	0	0	0	X
Cylindrovertilla kingi	0	0	0	0	0	X
Glyptopupoides egregia	0	0	0	0	0	X
Gastrocopta pediculus	0	0	0	0	0	X
Gastrocopta bifurcata	X	0	0	0	X	X
Pupisoma circumlitum	X	0	0	X	0	X
Pupisoma porti	X	0	X	0	0	X
Pupillidae MV1	X	X	0	0	0	X
Pupillidae MV2	X	0	0	0	0	0
**Lamellaxis clavulinus*	X	0	0	0	0	0
**Lamellaxis gracilis*	X	0	0	0	X	0
Strangesta ramsayi	0	X	X	0	0	0
Strangesta assimilans	0	0	0	X	0	0
Strangesta bullacea	0	0	0	0	X	0
Echotrida strangeoides	X	X	X	0	0	0
Saladelos macquariensis	0	0	0	0	0	X
Saladelos urarensis	0	0	0	X	0	0
Rhytididae MV1	0	0	0	0	0	X
Rhytididae MV2	0	0	0	0	0	X
Rhytididae MV3	X	X	X	0	0	X
Rhytididae MV7	0	0	0	0	0	X
Rhytididae NN1	0	0	0	X	0	0
Rhytididae BR1	X	X	X	0	0	0
Rhytididae SQ5	X	X	0	0	0	0
Hedleyella falconeri	0	X	X	0	0	X
Hedleyella maconelli	X	0	0	0	0	0
Brazieresta larreyi	0	0	0	0	0	X
Pedinogyra rotabilis	0	X	X	0	0	0
Iotula microcosmos	X	X	X	X	X	X
**Paralaoma caputspinulae*	0	0	0	0	0	X
Punctidae MV1	0	0	X	0	0	0
Ngairea corticicola	0	0	X	0	0	0
Setomedea seticostata	0	X	X	0	0	0
Mussonula verax	X	X	0	0	0	0
Nautiliropa omicron	X	X	X	0	0	0
Hedleyoconcha delta	0	0	X	0	0	X
Gyrocochlea vinitincta	0	0	X	0	0	0
Gyrocochlea convoluta	0	0	X	0	0	0

(continues)

(continued)

	Kenilworth	Brisbane	Lamington	Glenugie	Iluka	Kempsey
Gyrocochlea cinnamea	X	0	0	0	0	0
Gyrocochlea planorbis	0	0	0	0	0	X
Coenocharopa multiradiata	0	X	0	X	0	X
Coenocharopa sordidus	X	X	0	0	0	0
Coenocharopa parvicostata	X	X	0	0	0	0
Coenocharopa macromphala	X	0	0	0	0	0
Coenocharopa yessabahensis	0	0	0	0	0	X
Rotacharopa densilamellata	X	X	0	0	0	0
Allocharopa belli	X	0	X	0	0	X
**Discocharopa aperta*	X	0	0	0	X	X
Egilomen globosa	X	0	0	0	0	0
Egilomen lirata	0	0	0	0	0	X
Elsothera genithecata	0	0	X	0	0	0
Elsothera nautilodea	0	0	0	X	0	0
Letomola contortus	0	0	0	0	0	X
Rhophodon kempseyensis	0	0	0	0	0	X
Rhophodon minutissimus	X	0	0	0	0	0
Rhophodon colmani	X	0	0	0	0	0
Omphaloropa varicosa	X	0	0	0	0	0
species 1 indet.	0	X	0	0	0	0
Charopidae BR2	0	0	X	0	0	0
Charopidae BR11	X	0	0	0	0	0
Charopidae BR13	0	0	X	0	0	0
Charopidae BR15	0	0	X	0	0	0
Charopidae BR22	0	X	X	0	0	0
Charopidae BR23	0	X	0	0	0	0
Charopidae BR28	X	X	0	0	0	0
Charopidae BR29	0	X	0	0	0	0
Charopidae BR30	X	0	0	0	0	0
Charopidae BR31	X	0	0	0	0	0
Charopidae BB39	X	0	X	0	0	0
Charopidae BR41	0	0	X	0	0	0
Charopidae BR42	0	0	X	0	0	0
Charopidae BR43	0	0	X	0	0	0
Charopidae NN6	0	0	0	X	X	0
Charopidae NN7	0	0	0	X	0	0
Charopidae NN8	0	0	0	0	X	0
Charopidae NN17	0	0	0	X	0	0
Charopidae MV1	0	0	0	0	0	X
Charopidae MV3	0	0	0	0	0	X
Charopidae MV4	0	0	0	0	0	X
Charopidae MV7	0	0	0	0	0	X
Charopidae MV8	0	0	0	0	0	X
Charopidae MV11	0	0	0	0	0	X
Charopidae MV12	0	0	0	0	0	X
Charopidae MV13	0	0	0	0	0	X
Charopidae MV14	0	0	0	0	0	X
Charopidae MV16	0	0	0	0	0	X
Charopidae MV22	0	0	0	0	0	X
Charopidae MV24	0	0	0	0	0	x
Charopidae MV25	0	0	0	0	0	X

(continues)

(continued)

	Kenilworth	Brisbane	Lamington	Glenugie	Iluka	Kempsey
Charopidae MV26	0	0	0	0	0	X
Charopidae MV29	0	0	0	0	0	X
Charopidae MV30	0	0	0	0	0	X
Charopidae MV31	0	0	0	0	0	X
Charopidae MV35	0	0	0	0	0	X
Charopidae MV46	0	0	0	0	0	X
Charopidae NE3	0	0	0	0	0	X
Charopidae SQ4	X	0	0	0	0	0
Fastosarion aquila	X	X	X	0	0	0
Parmavitrina planilabris	0	0	0	0	0	X
Nitor medioximus	0	0	0	0	0	X
Nitor pudibunda	X	X	X	0	0	0
Nitor subrugata	0	0	X	0	0	0
Nitor graftonensis	0	0	0	0	X	0
Liardetia scandens	X	0	0	0	0	X
Coneuplecta calculosa	X	X	X	X	0	X
Tarocystis responsivus	X	0	0	0	0	0
species 2 indet.	0	X	0	0	0	0
Helicarionidae MV1	0	0	0	0	0	X
Helicarionidae MV2	0	0	0	0	0	X
Helicarionidae MV3	0	0	0	0	0	X
Helicarionidae MV4	0	0	0	0	0	X
Helicarionidae MV5	0	0	0	0	0	X
Helicarionidae NN5	0	0	0	X	0	0
Helicarionidae NN10	0	0	0	X	0	0
Helicarionidae BR1	0	0	X	0	0	0
Helicarionidae BR5	X	X	X	0	0	0
Helicarionidae BR7	X	X	0	0	0	0
Helicarionidae BR9	0	0	X	0	0	0
**Zonitoides arboreus*	0	0	0	0	X	0
**Bradybaena similaris*	0	0	0	0	0	X
Sphaerospira fraseri	X	X	X	X	0	0
Thersites novaehollandiae	0	0	0	0	0	X
Thersites richmondiana	0	X	X	0	0	0
Papuexul bidwilli	X	0	0	0	0	0
Posorites turneri	0	0	X	0	0	0
Posorites conscendens	0	X	X	0	0	X
Ramogenia challengeri	X	X	X	0	0	0
Austrochloritis separanda	X	0	0	0	0	0
Austrochloritis porteri	0	0	X	0	0	0
Austrochloritis nambucca	0	0	0	0	0	X
Camaenidae SQ2	X	X	0	0	0	0
Camaenidae BR1	0	0	X	0	0	0
Camaenidae NN1	0	0	0	X	0	0
Camaenidae NN2	0	0	0	0	X	0
Camaenidae MV4	0	0	0	0	0	X
Camaenidae MV6	0	0	0	0	0	X
Camaenidae MV10	0	0	0	0	0	X
Camaenidae MV11	0	0	0	0	0	X
total	49	34	41	17	11	67
unique	20	4	18	10	5	52
% unique	40.82	11.76	43.9	58.82	45.45	77.61

RESEARCH NOTE

MALACOLOGIA, 2007, 49(2): 465–470

NUPTIAL FEEDING IN THE FRESHWATER SNAIL *POMACEA CANALICULATA* (GASTROPODA: AMPULLARIIDAE)

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ABSTRACT

The males of the freshwater snail *Pomacea canaliculata* have a copulatory apparatus consisting in a delicate penis running through a muscular channeled sheath bearing three different glands. The outer gland is the most conspicuous one and often secrets a sticky drop of mucus during copula. However, its function is enigmatic because it opens toward the exterior of the pallial cavity. During laboratory trials performed to register copula duration and mating behaviour, we observed that females of *P. canaliculata* eat this secretion with approximately two deglutition events on average during the copula. This behaviour can be interpreted as a nuptial feeding, possibly functioning as an additional male mating effort to entice the female to remain in copula for long periods. To our best knowledge, there are no previously registered cases of nuptial feeding in gastropods.

Key words: *Pomacea canaliculata,* copula duration, apple snail, nuptial feeding, mating effort, paternal investment.

INTRODUCTION

Pomacea canaliculata (Lamarck 1822) is a South American freshwater snail that like other members of the genus has become an invader, promoting ecosystem changes in natural wetlands (Carlsson et al., 2004) and a serious rice pest in Asia (Cowie, 2002) during the last two decades. This gastropod is dioecious, oviparous, and shows a long lasting copula (10–18 h) and a multiple mating behaviour, at least under laboratory conditions (Andrews, 1964; Albrecht et al., 1996).

Males have a complex copulatory apparatus that occupies the roof margin of the pallial cavity and consists of a muscular penis sheath with a deep channel on its inner face along which the penis runs during copula (Andrews, 1964; Gamarra-Luques et al., 2006). The penis sheath presents three macroscopic glands: the apical gland, the marginal gland (on the right margin of the channel), and the outer gland (on the basal portion). The outer sheath gland is the most bulky and conspicuous, showing an external slit-like opening slightly to the left of the medial sheath line. The secretion of the apical and marginal glands apparently acts in penis sheath adhesion and in the lubrication of the penis (Andrews, 1964), but the function of the outer sheath gland is enigmatic because it opens toward the exterior of the pallial cavity. During copula, the outer sheath gland often secrets a sticky drop of mucus (Andrews, 1964; Berthold, 1991). Andrews (1964) suggested that this secretion could act as a repellent for the males that remain near the mating couple. The aim of our paper is to report laboratory observations that clarify the functional role and the evolutive significance of this gland and its secretion in the mating behaviour of this snail.

METHODS

The experimental snails (males: 31–43 mm shell length, females: 41–49 mm) were brought to the laboratory in March 2005 from Curamalal stream, Buenos Aires Province, Argentina (37°14'31"S, 62°08'04"W), kept in a rearing room at 25 ± 2°C, under 14 h light/day and fed with lettuce *ad libitum.* Snails were sexed by the shape of the shell aperture and operculum (Estebenet et al., 2006). Laboratory trials were performed in 3 litre glass aquaria with water at 24–26°C and permanent

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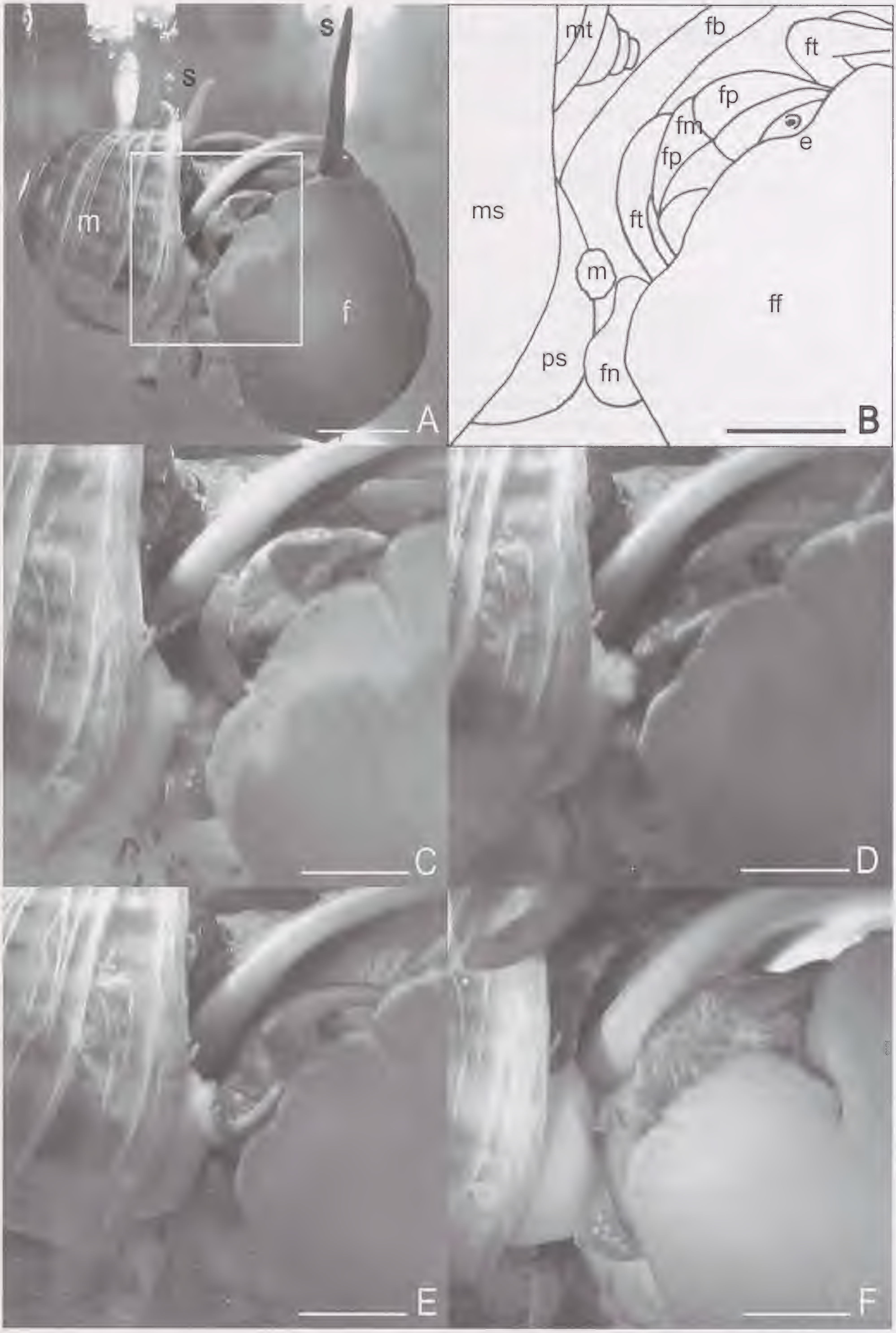
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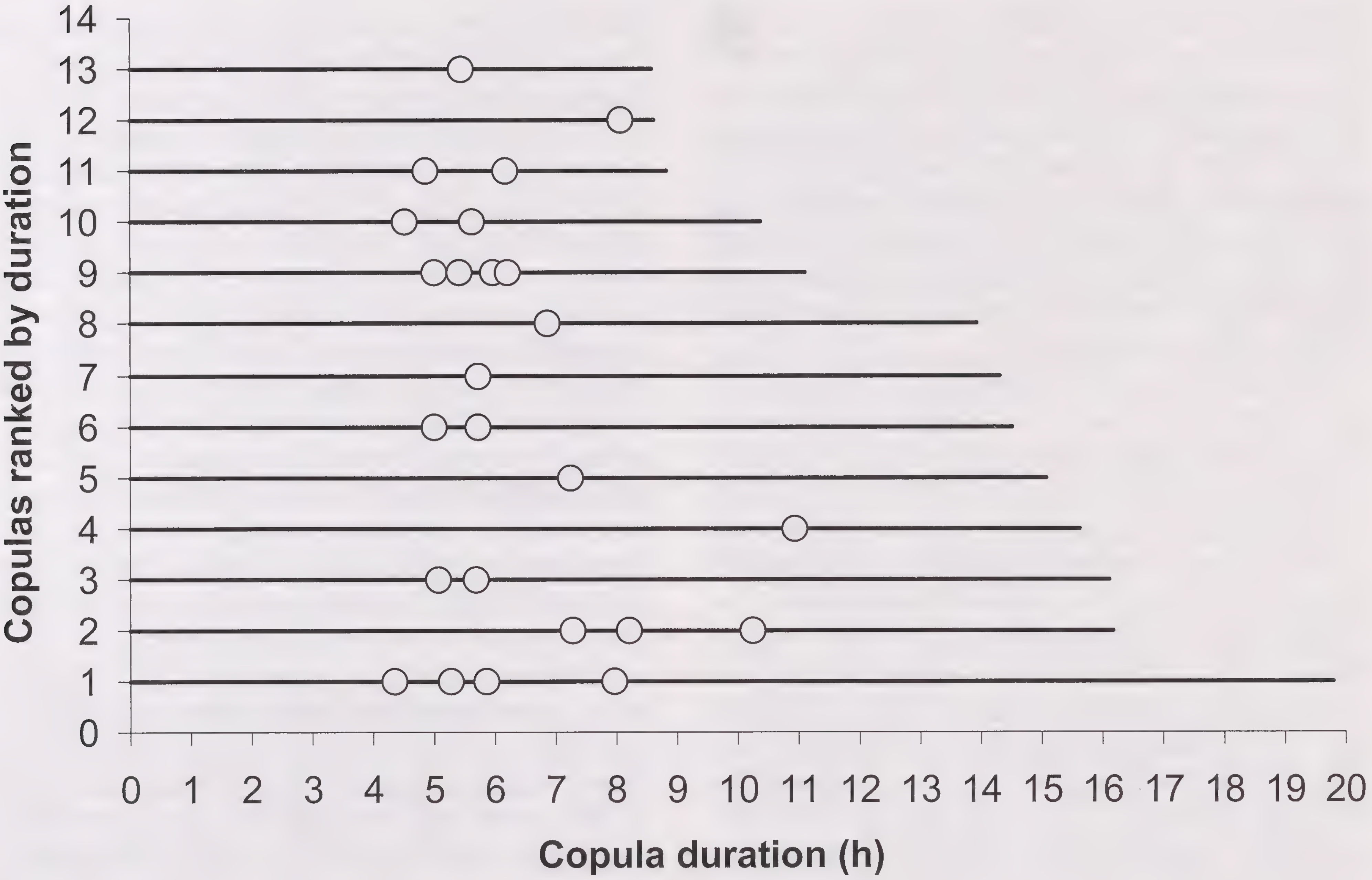

FIG. 2. Scheme showing the different copula duration and the deglutition events observed. Each bar represents one copula and its length the duration in hours. The circles indicate each mucus deglutition event.

illumination in order to observe the mating behaviour of isolated couples. The snails were observed continuously for 24 h in order to register the start, the end and any particular copula behaviour. During the copula the females remain mostly attached to or crawling on the aquarium walls, thus allowing the observation of the mating couple. We considered that the copula had begun when the male inserted the penis sheath into the female cavity, because it is seldom possible to observe the penis passing through the penis sheath channel (as described by Andrews, 1964).

RESULTS

During the courtship the male mounts the female adhering his foot to her last shell whorl, generally oriented toward the right side of her aperture (Fig. 1A). The male usually remains in this position up to 2 h. The female generally shakes her shell when the male adopts a closer position, unfolding his penis sheath to insert it into the female pallial cavity. If the female does not shake the male off, the copula starts. During most of the process both partners remain motionless with their heads par-

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FIG. 1. Different stages in a mucus deglutition event as seen through the wall of an aquarium (water surface is located towards the upper side of all pictures). A: Typical mating position (female on the right and male on the left) where it is possible to observe a mucus droplet released from the outer penis sheath gland opening; f, female; m, male; s, siphon; scale bar = 20 mm; B: Detailed scheme of the frame in A. fm, female mouth; ff, female foot; fb, female mantle border; ft, female tentacle; fn, female nucal lobule; fp, female palp; e, female eye; ms, male shell; mt, male left tentacle; ps, basal portion of the penis sheath; m, mucus droplet; scale bar = 10 mm; C: First moment in mucus droplet formation; scale bar = 10 mm; D: Detail showing the female turning her head towards the mucus droplet; scale bar = 10 mm; E: Detail showing the mucus deglutition; scale bar = 10 mm; F: End of deglutition event showing the depressed area of the outer sheath gland opening; scale bar = 10 mm.

tially withdrawn, commonly with their cephalic tentacles coiled (Figs. 1A, B). Females can move freely and feed during the copula, but males cannot and depend on the female's movements to go near the water surface to ventilate the aerial lung. The copula ends when the male withdraws his penis sheath, but then he often remains stuck onto the female's shell for around an hour longer with his penis sheath folded and motionless.

During the copula duration trials, we observed various males of *P. canaliculata* releasing a pale salmon coloured mucus from their outer sheath gland opening. Before the secretion begins, the outer sheath gland swells and its opening becomes evident as a depressed area. The secretion seems to be steadily extruded through the opening forming a mucus droplet (Fig. 1C) that is swallowed at irregular intervals by the female. The female turns round her head toward the gland opening (Fig. 1D), expanding her snout and picking up the droplet with her mouth (Fig. 1E) and slowly swallowing it, without the radular movements typical of grazing or ciliary feeding (Cazzaniga & Estebenet, 1984). After each deglutition event, it is possible to observe the depressed slit-like opening of the outer sheath gland before the accumulation of a new droplet (Fig. 1F). These observations of secretion and deglutition events were only possible when the male was loosely attached to the female.

Thirteen out of the 32 copulas observed in the laboratory showed at least one deglutition event, summarising 25 deglutition events (Fig. 2). We observed 1.9 ± 1.12 deglutition events per copula (mean ± S.D.), each of them lasting 5–15 seconds. The mean copula duration observed was 13.31 ± 3.5 h, and the first mucus deglutition was recorded approximately in the middle of the copula (6.18 ± 1.86 h). Out of a set of 56 copula (13 mentioned above and 43 additional copula trials) in which mucus secretion was observed, in only two cases it was not eaten by the female and the mucus formed a thick thread instead of a droplet.

DISCUSSION

Our observations on mating behaviour are in agreement with the descriptions of Andrews (1964) and Albrecht et al. (1996) for *P. canaliculata* from other geographical origins. The mucus release from the outer sheath gland seems to be common, because we have observed it in many mating couples, both in the laboratory and in the field. We believe that the deglutition of this secretion had been previously unnoticed because it is difficult to observe the exact moment in which it takes place. On one hand, the deglutition event occurs in a few seconds and, although the mucus droplet formation can last up to 25–30 minutes, it becomes visible only when it reaches a diameter of 2 mm or more. On the other hand, the deglutition events are often difficult to observe when the male is tightly adhered to the female and his head or the basal portion of the penis sheath are not visible. The female right nucal lobule often hides the basal portion of the penis sheath, blocking the outer sheath gland opening, but we do not know if in these cases it interferes with mucus secretion and deglutition or merely with its observation.

Andrews (1964) and Berthold (1991) had already observed the mucus release in laboratory copulas of *P. canaliculata*, but they never mentioned that the female feeds on it. Berthold (1991) also described the mucus secretion from the external sheath gland in males of *Marisa cornuarietis* (Linné, 1767) but he did not discuss anything about its function. Andrews (1964) reported the mucus release only when a group of males remains near the mating partners, suggesting that it could be acting as a repellent for prowler males. Now this explanation seems unlikely, because the mucus release occurs even in the absence of prowler males.

Nuptial feeding comprises any form of nutrient transference from the male to the female during or directly after courtship or copulation (Vahed, 1998), a definition suitable for the behaviour observed in *P. canaliculata*. Nuptial feeding often takes the form of food gifts, the nutrients being transferred in seminal fluids or spermatophores, as prey gifts captured or collected by the male or as somatic gifts (male's body or male glandular secretions) (Andersson, 1994; Vahed, 1998). To our best knowledge, there are no previously registered cases of this behaviour in gastropods. Love dart shooting in helicids has been considered as a calcium gift to the recipient partner, although currently it is interpreted as a case of reproductive manipulation (Pomiankowski & Reguera, 2001).

Two non-mutually exclusive hypotheses for the possible functions of nuptial feeding have been discussed (Vahed, 1998). In the first, it acts as a paternal investment, and the male increases his fitness by benefiting mainly his own offspring with the nutrients transferred. In the second, it is part of the male mating effort acting in female attraction, female mating acceptance or in the lengthening of copula to maximise the sperm transference.

Pomacea canaliculata females are able to store the ejaculates in different pouches keeping the sperm fertile for up to 140 days (Estebenet & Martín, 2002). Sperm mixing takes place inside the seminal receptacle of *P. canaliculata* females, and double paternity occurs in a single spawn (Yusa, 2004). The sperm mixing and the possibility of cryptic female choice reduce the chances that a given male can benefit his own offspring through his nuptial gift. Although the mucopolysaccharides detected in the outer sheath gland of *P. canaliculata* (Andrews, 1964) and *M. cornuarietis* (Schulte-Oehlmann et al., 1994) could be nutritious and easily assimilated, the amount transferred is minimal, since each droplet is smaller and lighter than a single egg of *P. canaliculata*, and females can spawn 4,500 eggs on average during their lifetime (Estebenet & Martín, 2002). This makes less plausible the paternal investment explanation in *P. canaliculata*.

On the other hand, the number of eggs spawned by virgin females after a single copula is directly related to the copula duration (unpublished results). The nuptial feeding observed in *P. canaliculata* could be an additional mating effort to entice the female to remain in copula for long periods. Given the possibility of sperm mixing, this strategy could improve the competitive ability of a given male by increasing his sperm numbers relative to other's inside the female reproductive tract (sperm loading hypothesis, García-González & Gomendio, 2004).

Notwithstanding the advances in the knowledge of their reproductive anatomy (Berthold, 1991; Schulte-Oehlmann et al., 1994; Gamarra-Luques et al., 2006), for most ampullariid species details of mating behaviour are unknown. Albeit anatomically diverse, a presumably homologous outer sheath gland is present in all the Neotropical genera – *Pomacea, Marisa, Pomella, Asolene, Surinamia and Felipponea* – and in the Old World genus *Pila* (Berthold, 1991; Bieler, 1993). Probably the deglutition of the mucus from the outer sheath gland reported here for *P. canaliculata* is not an exception in the genus or the family, though a range of complexity in nuptial feeding behaviour can certainly be expected.

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